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

SEP 15 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Diazinon Registration Standard Followup: Response to Residue Chemistry Data Requirements (MRID Nos. 41119201, 41072601, 41072602, DEB Nos. 5452, 5294, HED Project Nos. 9-1626, 9-1389, RD Record No. 244503).

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Attached is a review of a followup to the Diazinon Registration Standard prepared by the Dynamac Corp. under supervision of the Dietary Exposure Branch (DEB). This review has undergone secondary review and revision in the Dietary Exposure Branch and reflects current Branch policies.

If you need additional input, please advise us.

cc with Attachment: PMSD/ISB, RF, Circu, Diazinon Standard File (Boodee), E. Haeberer

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DYNAMAC
CORPORATION

Final Report

DIAZINON
Task 4: Registrant's Response to Residue Chemistry Data Requirements

August 1, 1989

Contract No. 68-D8-0080

Submitted to:
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Arlington, VA 22202

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DIAZINON

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

Task - 4

BACKGROUND

The Diazinon Residue Chemistry Chapter dated 8/6/86 and a Data Call-In (DCI) Notice dated 5/1/87 conclude that adequate analytical methodologies are available to detect residues of parent diazinon and the metabolites diazoxon (G-24576), hydroxydiazinon (CGA-14128), and the hydroxypyrimidine metabolite of diazinon (G-27550) in or on plant commodities and in animal products. However, it was stated that if the required plant and animal metabolism studies reveal additional metabolites of toxicological concern, appropriate analytical methodologies for these compounds will be required. Ciba-Geigy Corp. submitted gas-liquid chromatographic (GLC) analytical method AG-550 validated for quantifying diazinon, G-24576, and CGA-14128 (1989; MRID 41072601, DEB No. 5294), and stated the intention to use this method in all Ciba-Geigy residue studies. We note that data on animal metabolism have been submitted and reviewed in an Agency memorandum by R.B. Perfetti (DEB Nos. 4773, 4774, 4778, 4779, and 4880, dated 2/8/89), who concluded that the requirement for a ruminant metabolism study had been fulfilled and that the residues in milk and tissues consist of G-27550 and 2-(1-hydroxyisopropyl)-4-methyl-6-hydroxypyrimidine (GS-31144) in addition to the compounds quantified by method AG-550; we defer to Toxicology Branch (TB) as to the significance of these additional metabolites.

As required by the Agency, Ciba-Geigy Corp. has submitted data depicting the recovery of diazinon and metabolites using FDA Multiresidue Protocols I-IV published in Pesticide Analytical Manual (PAM), Vol. I (1989; MRID 41072602).

Data Requirements for Diazinon:

The Diazinon Guidance Document dated December, 1988 concluded that additional analytical methods, validation data, and residue data (for representative commodities) are required if the metabolism studies requested in 171-4 (Nature of the Residue in Plants and Nature of the Residue in Animals) indicate that additional metabolites constitute residues of toxicological concern in plants or animals.

CONCLUSIONS

1(a). The capillary GC/FPD analytical procedure (analytical method AG-550) submitted (1989; MRID 41072601) is adequate for the determination of diazinon, hydroxydiazinon (CGA-14128), and diazoxon (G-24576) residues in or on plant commodities and in animal products. The detection limit of the method is 0.005 ppm,

for individual reference standards, and recovery of diazinon and each of its metabolites from various plant and animal samples fortified at 0.01-25 ppm ranged from 63 to 134%.

1(b). Additional analytical methodology may be required if TB determines that G-27550 or GS-31144 in milk and ruminant tissues is of concern or if the required plant and poultry metabolism studies reveal additional residues of toxicological concern.

2. Multiresidue Protocol III, using 2% DEGS and OV-101 GLC columns, is the most appropriate for the determination of diazinon, diazoxon (G-24576), G-27550, and hydroxydiazinon (CGA-14128) in plant commodities and animal tissues. None of the Multiresidue Protocols submitted are adequate for the determination of the metabolite GS-31144 due to its poor GLC profile and poor sensitivity to GLC detection and HPLC/fluorescence detection. The test data will be forwarded to FDA for evaluation/inclusion in a future updating of PAM I.

RECOMMENDATIONS

Ciba-Geigy Corp. should be notified that an appropriate analytical method for the determination of the metabolite GS-31144 in or on plant commodities and in animal products may be required. In addition, although the requirement for a ruminant metabolism study has been fulfilled (EPA memo from R.B. Perfetti dated 2/8/89), the requirements for a poultry metabolism study and plant metabolism data remain outstanding. If the required plant and poultry metabolism studies reveal additional metabolites of toxicological concern, appropriate analytical methodologies for these compounds will be required.

DETAILED CONSIDERATIONS

GLC Analytical Method AG-550

Ciba-Geigy Corp. (1989; MRID 41072601) submitted a capillary column GLC method (method no. AG-550) for determination of diazinon, CGA-14128, and G-24576 residues in or on plant commodities and in animal products. Residues in plants, animal tissues (except beef fat), milk, and eggs are extracted with acetone:water (90:10, v/v). The extracts are filtered, partitioned with petroleum ether:deionized water:methylene chloride (50:10:50), and the aqueous phase is mixed with sodium chloride and partitioned into methylene chloride. The organosoluble fractions are combined, filtered through anhydrous sodium sulfate, and evaporated to dryness. The extract is redissolved in acetone and analyzed by GLC using a capillary column (DB1701 column) with flame photometric detection (FPD). Residues are quantified by comparing the peak responses of known concentrations of standard solutions of diazinon, CGA-14128, and

G-24576 to the responses produced by residues extracted from individual samples. In addition, if interference with the elution of diazinon occurs, the evaporated residue is dissolved in petroleum ether and cleaned up on a Florisil Sep-Pak column prior to GLC analysis. Samples of fortified corn oil are dissolved in hexane and extracted with acetonitrile saturated with hexane. The acetonitrile fraction is evaporated to dryness, redissolved in acetone, and residues are quantified by capillary column GLC/FPD, as described previously.

Residues in beef fat are extracted with hexane and the extract is filtered and partitioned into acetonitrile. The acetonitrile fraction is evaporated to dryness, redissolved in acetone, and partitioned with petroleum ether:deionized water:methylene chloride (50:10:50). The aqueous phase is mixed with sodium chloride and partitioned into methylene chloride. The organosoluble fractions are combined, filtered through anhydrous sodium sulfate, and evaporated to dryness. The extract is redissolved in acetone and residues are quantified by capillary column GLC/FPD, as described previously. Diazinon residues in or on plant commodities and in animal products may be confirmed by GLC using an alternate capillary column (CP-WAX column) or a packed column (2% DEGS column), and/or by using an alternate mode of detection (nitrogen/phosphorous detection).

Validation data for this method are summarized below in Table 1. The method detection limit is 0.005 ppm (0.01 ng/2 μL) for individual reference standards. Apparent residues in control samples ranged from <0.005 ppm (nondetectable) to 0.057 ppm.

Table 1. Recovery of diazinon, G-24576, and CGA-14128 from fortified samples of plant commodities and animal products using capillary column GLC/FPD method no. AG-550.

Commodity	Fortification (ppm)	Percent Recovery		
		Diazinon	G-24576	CGA-14128
Tomatoes	0.01	90	100	90
	0.05	100	97	90
Apples	0.01	89	84	72
	0.5	95	91	87
Carrots	0.01	80	90	90
	1.0	80	93	92
Cucumbers	0.01	126	105	72
	25.0	92	91	112
Peas	0.01	116	100	87
	0.5	104	103	109

(continued)

Table 1. (Continued)

Commodity	Fortification (ppm)	Percent Recovery Diazinon	G-24576	CGA-14128
Peppers	0.01	94	120	110
	0.5	105	110	95
Potatoes	0.01	121	119	115
	0.1	108	105	107
Radishes	0.01	114	94	88
	0.5	111	107	111
Cabbages	0.01	122	115	106
	1.0	106	104	102
Squash	0.01	117	107	116
	1.0	99	98	100
Broccoli[a]	0.01	85	100	90
	1.0	74	103	100
Onions[a]	0.01	94	90	97
	1.0	73	90	92
Green Beans	0.01	75	103	104
	0.05	117	116	116
Raspberries	0.01	101	280	109
	0.5	113	89	107
Crude Corn	0.01	116	114	80
Oil[b]	1.0	92	84	92
Refined Corn	0.01	108	118	90
Oil[b]	1.0	94	84	93
Ref., Bleached	0.01	119	90	63
Corn Oil[b]	1.0	101	85	90
Almonds	0.01	108	83	93
	0.5	85	77	81
Beef Liver	0.01	112	64	102
	1.0	94	80	96
Beef Fat	0.01	96	134	121
	1.0	105	109	106
Milk	0.01	87	110	113
	1.0	122	112	126
Poultry Lean	0.01	91	98	98
Meat	1.0	98	79	96
Eggs	0.01	70	123	103
	1.0	92	102	98

[a] Diazinon residues determined following Florisil Sep-Pak cleanup; G-24576 and CGA-14128 residues determined prior to cleanup.

[b] Diazinon residues determined following dilution of GC solution; diazoxon and hydroxydiazinon residues determined prior to dilution of GC solution.

Recovery of Diazinon and Metabolites Using Multiresidue Protocols

Ciba-Geigy Corp. (1989; MRID 41072602) submitted data pertaining to the determination of diazinon and its metabolites G-24576, CGA-14128, GS-31144, and G-27550 in or on plants in animal tissues using FDA Multiresidue Protocols I, II, III, and IV (report no. ABR-88125). Fortified samples are extracted and prepared for analysis by GLC according to the procedures referenced in EPA Multiresidue Protocols I-IV and the FDA Pesticide Analytical Manual (PAM), Vol. I.

Protocol I:

Three stationary phases, 5% OV-101, 3% OV-225, and 3% OV-17 were evaluated for determination of each test compound. The conditions for each column were adjusted to give the appropriate retention times of chlorpyrifos and p,p-DDT. Attenuation was selected to give a 50% full-scale deflection (FSD) response for injection of 1.5 ng of chlorpyrifos. The chromatographic profiles of the test compounds are illustrated in Table III of the submission, reproduced below.

Relative retention times and sensitivity results for diazinon and metabolites - Protocol I using EC detection.

GC Column	Compound	RRc	ng for 50% FSD
5% OV-101	Diazinon	0.518	3.0
	G-24576	0.461	18.0
	G-27550	-	ND
	CGA-14128	0.703	2.0
	GS-31144	0.303	220.0
3% OV-225	Diazinon	0.410	4.5
	G-24576	0.558	60.0
	G-27550	-	ND
	CGA-14128	0.800	2.0
	GS-31144	-	ND
3% OV-17	Diazinon	0.440	3.0
	G-24576	0.446	30.0
	G-27550	-	ND
	CGA-14128	0.663	0.6
	GS-31144	0.208	750.0

ND - Not detectable at 1000 ng injection limit.

Each compound was tested for recovery through a Florisil cleanup column conditioned and calibrated to provide the appropriate elution of heptachlor epoxide and eldrin as specified in Protocol I. Florisil columns were loaded with standard mixtures

containing 10 ug of diazinon, 10 ug of G-27550, 50 ug of G-24576, 100 ug of CGA-14128, and/or 100 ug of GS-31144. Eluates were analyzed by GLC using 5% OV-101 or 3% OV-225 columns. Recovery of diazinon was 90-116% from Florisil eluted with 15% diethyl ether/petroleum ether and 80-88% with 50% dichloromethane/1.5% acetonitrile/48.5% hexane. CGA-14128 recovery was inadequate (9-22%) using 50% diethyl ether/petroleum ether. None of the other metabolites was recovered from florisil. Thus, only diazinon was subjected to further testing under Protocol I.

Samples of tomatoes, corn grain, poultry lean meat, and beef liver were fortified with diazinon at 1 and 2x the tolerance levels. Samples were extracted and cleaned up on a Florisil PR column and residues are quantified by GLC using a 5% OV-101, 3% OV-17, or 3% OV-225 column with electron capture detection (ECD). The conditions that gave recoveries ≥30% are summarized below in Table 2.

Table 2. Recovery of diazinon from commodities using multiresidue Protocol I (cleanup on mixed ether Florisil column unless otherwise specified).

Commodity	Fortification (ppm)	Elution Solvent[a]	Column	Recovery (%)
corn grain	0.7	6% DE/PE	OV-101	48-62
	1.4	6% DE/PE	OV-101	25-36
	0.7	6% DE/PE	OV-225	48-68
	1.4	6% DE/PE	OV-225	24-30
beef tenderloin	0.75	PE	OV-101	66-70
	1.5	PE	OV-101	69
	0.75	PE	OV-225	71-72
	1.5	PE	OV-225	70-74
(dichloromethane	0.75	PE	OV-101	67-69
Florisil	1.5	PE	OV-101	74-81
cleanup)	0.75	PE	OV-225	73-78
	1.5	PE	OV-225	80-82
tomatoes	0.7	15% DE/PE	OV-101	82-86
	1.4	15% DE/PE	OV-101	73-86
	0.7	15% DE/PE	OV-225	80-98
	1.4	15% DE/PE	OV-225	76-87
beef liver	0.7	15% DE/PE	OV-101	64-73
	1.4	15% DE/PE	OV-101	75-88
	0.7	15% DE/PE	OV-225	84-89
	1.4	15% DE/PE	OV-225	79-94

[a]DE/PE = diethyl ether/petroleum ether.

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Protocol II:

The chromatographic profiles of the test compounds were determined on four stationary phases, 5% OV-101, 3% OV-17, 2% DEGS, and Ultrabond 20 SE, using flame photometric (FP) and nitrogen/phosphorous detection (N/P). Standard solutions of diazinon, G-24576, CGA-14128, GS-31144, and G-27550 were cleaned up on a charcoal/magnesium oxide/celite column and residues were quantitated by GLC. The recoveries on the OV-101 and OV-17 columns are summarized below in Table 3.

Table 3. Recovery of diazinon and its metabolites using multiresidue Protocol II following cleanup on a charcoal/magnesium oxide/celite column.

Compound	Percent Recovery	
	OV-101 Column	OV-17 Column
Diazinon	64, 98	69, 96
G-24576	48, 62	47, 64
G-27550	0	0
CGA-14128	80, 86	76, 88
GS-31144	0	0

Diazinon, G-24576, and CGA-14128 were recoverable at >30%; G-27750 and GS-31144 were not detectable. The test compounds were subjected to Protocol III procedures before completing Protocol II to determine the need for charcoal cleanup of fortified substrates.

Protocol III: The column stationary phases and detectors evaluated were the same as those used in Protocol II. The conditions for the 2% DEGS and Ultrabond 20 SE were adjusted to give the retention times of chlorpyrifos, parathion, and methamidophos specified in PAM, Vol. I. The OV-101 and OV-17 columns were calibrated with chlorpyrifos alone. Detector sensitivity was adjusted to give 50% FSD with 1.5 ng of chlorpyrifos. The chromatographic profiles of the test compounds are illustrated in Tables XVIII and XIX of the submission, reproduced below.

Relative retention times and sensitivity results for diazinon and metabolites on the gas chromatography columns used with N/P detection - Protocol III.

GC Column	Compound	RRc	ng for 50% FSD
5% OV-101	Diazinon	0.573	0.40
	G-24576	0.522	0.60
	G-27550	0.280	3.0
	CGA-14128	0.788	2.0
	GS-31144	0.470	100.0
2% DEGS	Diazinon	0.384	0.30
	G-24576	0.579	0.60
	G-27550	0.725	3.0
	CGA-14128	1.09	1.5
	GS-31144	2.32	25.0
3% OV-17	Diazinon	0.474	0.25
	G-24576	0.494	0.60
	G-27550	0.294	3.0
	CGA-14128	0.702	2.0
	GS-31144	0.469	100.0
Ultrabond 20SE	Diazinon	0.488	0.20
	G-24576	0.582	0.45
	G-27550	0.458	0.5
	CGA-14128	0.949	1.5
	GS-31144	4.52	100.0

Relative retention times and sensitivity results for diazinon and metabolites - Protocol III using FP detection.

GC Column	Compound	RRc	ng for 50% FSD
2% DEGS	Diazinon	0.400	0.6
	CGA-14128	1.06	2.0
	G-24576	0.601	1.5
	G-27550	-	ND (>1μg)
	GS-31144	-	ND (>1μg)

ND - Not detectable.

Using N/P detection, all five compounds were detectable below the injection limit of 1,000 ng specified in PAM, Vol. I. The 2% DEGS column provided the best responses with 0.3-25 ng providing 50% FSD; this column and OV-101, judged as the most suitable alternate, were chosen for substrate fortification tests.

Extracts of fortified samples were analyzed using GLC-N/P. G-31144 was not recovered from fortified samples on either column. G-27550 is determined using a 2% DEGS column and all other

compounds are determined using a OV-101 column, with the exception of G-24576 residues in beef liver which are determined using a 2% DEGS column with flame photometric detection. The results of these tests are summarized below in Table 4.

Table 4. Recovery of diazinon and its metabolites from commodities using multiresidue Protocol III.

		Percent Recovery				
Commodity	ppm[a]	Diazinon[b]	G-24576[b]	G-27550[c]	CGA-14128[b]	GS-31144[b]
Tomatoes	0.7	94,109	77,83	99,105	120,125	ND[d]
	1.4	93,99	83,79	107,91	100,122	ND
Corn Grain	0.7	99,73	40,75	41,39	91,97	ND
	1.4	102,71	76,73	39,39	29,101	ND
Poultry	0.75	77,100	76,69	82,108	111,102	ND
Lean Meat	1.5	97,75	83,78	88,78	105,110	ND
Beef Liver	0.75	93,93	29,56[e]	107,92	90,81	ND
	1.5	92,92	3,9[e]	73,84	90,96	ND

[a] Fortification level.
[b] Residues determined using a OV-101 column with N/P detection.
[c] Residues determined using a 2% DEGS column with N/P detection.
[d] Not detected.
[e] Diazoxon residues in beef liver determined using a 2% DEGS column with flame photometric detection.

Protocol IV: Standard solutions of diazinon, G-24576, CGA-14128, GS-31144, and G-27550 are analyzed by HPLC using fluorescence detection and by HPLC with post-column OPA derivatization and fluorescence detection. Reagents for the OPA derivatization are prepared according to the method of Krause (1985). None of the standard compounds were detectable using Multiresidue Protocol IV analytical methods.

In summary, the analytical methods of Protocol I are adequate for the determination of diazinon residues only. Protocol II methods are adequate for the analysis of standard solutions of diazinon and CGA-14128; however, the charcoal cleanup procedures of this method are not necessary. Protocol IV HPLC methods are not adequate for the determination of diazinon and its metabolites.

Multiresidue Protocol III, using 2% DEGS and 5% OV-101 columns, is the most appropriate method for the determination of diazinon, diazoxon (G-24576), G-27550, and hydroxydiazinon (CGA-14128) in plant commodities and animal tissues. None of the Multiresidue Protocols are adequate for the determination of the metabolite GS-31144 due to its poor GLC profile and poor sensitivity to GLC detection and HPLC/fluorescence detection. The test data will be forwarded to FDA for evaluation/inclusion in a future updating of PAM I.