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Vinclozolin Decay on Different Grapevines in Four Differing Italian Areas

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A comparative study of the decay of Vinclozolin on four different grapevines in four widely differing areas of Italy was carried out. The residues from two different doses of the commercial product were examined by gas chromatography during 3 weeks following each treatment. The decline of the residues in all cases was described by an exponential trend ($Y = Ae^{-KX}$). The theoretical half-life of the original residue (that present after 2 h) varied quite considerably in the four areas (Piedmont, Friuli, Latium, Emilia), whereas successive treatments in the same location varied less. The minimum half-life was 1.2 days for the normal and 2.0 days for the double dose (Emilia); the maximum was 4.6 and 4.9 days (Piedmont).

INTRODUCTION

Vinclozolin, 3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione, is widely used in Italian grape cultivation to control *Botrytis* and has been the object of numerous studies from the purely residual point of view (Molinari et al., 1978, 1983; Barbina Taccheo et al., 1978; Zanini et al., 1980; Del Re et al., 1980, 1981; Flori et al., 1980, 1982; Cabras et al., 1983). One aspect has been rather neglected: the variations of the residues in the treatment parts of the plant induced by the environment. This investigation is important from both an agricultural and a pesticide residue level point of view.

It is normal practice both in Italy and in other countries to establish an interval of time after the final spraying with a pesticide at the end of which the residue in the edible product must not exceed a certain tolerance level. This is fixed for each pesticide without taking into account that it might differ widely from one environment to another, from one crop to another, or even from one variety to another of the same crop. The safety interval, moreover, is fixed with reference only to the final spraying and does not take into account the fact that the final residue level of an active principle is very often the consequence of several successive treatments carried out according to a time table or in response to particular environmental conditions or particular stages of development of the crop: each treatment could be influenced by those preceding it. On the other hand, the efficacy of many pesticides, especially if systemic or translaminal, is often a function of their persistence. This characteristic can be of extreme practical importance in preventive pest control and when protection is guaranteed only if the pesticide does not decay too rapidly.

With the same crop in different environments the effects of sunlight, daily temperature changes, and rainfall must be carefully evaluated by comparing the degradation curves. The present study was conducted to illustrate the varying behavior of a pesticide: widely differing environments were chosen (Piedmont, Friuli, Latium, Emilia), involving one crop, grapevine, and a single active principle, Vinclozolin, in a sample year, 1983, so as to illustrate a representative situation that can be generalized to a wider context.

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MATERIALS AND METHODS

The investigation was carried out during 1983 by the following research units of the Gruppo di Ricerca Italiano Fitofarmaci e Ambiente (Italian Group for Pesticide and Environment Research): A. Piedmont, Istituto di Chimica Agraria and Laboratorio NMR e Spettroscopie Applicate alla Tossicologia, University of Turin; Experimental sta-

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Table I. Experimental Conditions at Each Research Unit

	laboratory U			
	A: Piedmont	B: Friuli	C: Latium	D: Emilia
locality	Loazzolo (Asti)	Risano (Udine)	Aprilia (Latina)	Granarolo (Bologna)
variety of wine	Moscato Bianco	Pinot Bianco	Malvasia di Candia	Sangiovese
spraying period				
veraison	7/7/83	6/30/83	7/6/83	7/4/83
closing of bunches	8/18/83	7/26/83	8/8/83	8/10/83
21 days before harvesting	8/31/83	8/22/83	9/1/83	8/31/83
area of vineyard, m^2	4000	3600	6000	4000
aspect	hill W-NW	hill E-SE	plain	plain
rows in trials	4 × 2	3 × 2	4 × 2	4 × 1
period of sampling: leaves (days after spraying)				
1st spraying	0.08, 1, 2, 5, 12, 21	0.08, 1, 2, 4, 7, 22	0.08, 1, 2, 5, 12, 22	0.08, 1, 2, 21
2nd spraying	0.08, 1, 2, 5, 12, 13	0.08, 1, 2, 5, 12, 22	0.08, 1, 2, 5, 14, 22	0.08, 1, 2, 12
3rd spraying	0.08, 1, 2, 5, 12, 21	0.08, 1, 2, 4, 11, 21	0.08, 1, 5, 12, 26	0.08, 5, 12, 21
sampling of grapes after 3rd spraying (days after spraying)	0.08, 12, 21	21	0.08, 5, 26	12, 21
size of sample				
no. of leaves	20	20	20	20
kg of grapes	2	2	2	1

Table II. Metereological Data for Each Experimental Station during the Period June–September 1983

	station A				station B				station C				station D			
10-day period	I	II	III	monthly mean	I	II	III	monthly mean	I	II	III	monthly mean	I	II	III	monthly mean
June																
t_{min}, °C	14	12	14	13	15	11	14	13	10	12	13	12				
t_{max}, °C	28	26	27	27	31	25	27	25	30	28	30	28				
t_x, °C	21	19	21	20	23	18	20	19	26	19	20	22				
rel humidity, %	78	88	88	86					71	72	74	72				
rainfall, mm	1	80	26		9	28	8		0	108	12					
days of rain	2	3	4		1	2	1		0	3	4					
July																
t_{min}, °C	17	19	21	19	16	17	19	17	18	15	17	16	12	14	14	13
t_{max}, °C	30	34	35	33	32	33	32	32	30	33	36	33	32	38	38	36
t_X, °C	24	27	28	26	24	25	26	25	25	24	26	24	22	26	26	24
rel humidity, %	82	77	80	79					80	73	76	76	65	49	55	57
rainfall, mm	6	1	1		20	11	20		36	0	0		10	0	0	
days of rain	3	1	3		1	1	1		4	0	0		5	0	0	
August																
t_{min}, °C	15	15	12	13	15	16	17	16	13	17	17	14	12	10	16	13
t_{max}, °C	30	30	33	32	27	31	30	29	32	30	28	29	36	31	33	33
t_X, °C	22	22	23	22	21	24	24	23	19	23	25	23	24	20	20	22
rel humidity, %	70	75							69	79	84	79	51	56	71	60
rainfall, mm	22	1	30		33	0	11		0	32	77		10	7	9	
days of rain	1	6	11		2	0	1		0	3	4		3	3	3	
September																
t_{min}, °C					15	12	12	13	15	10	13	12	10	7	6	8
t_{max}, °C					27	25	28	27	29	26	27	27	30	29	28	29
t_X, °C					21	24	20	23	22	18	20	20	20	16	17	17
rel humidty, %									80	75	74	77	65	58	60	60
rainfall, mm					42	6	0		8	11	38		1	1	0	
days of rain					2	1	0		1	4	1		1	2	0	

tion, Loazzolo, province of Asti. B. Friuli, Centro Sperimentazione Agraria Friuli-Venezia Giulia; experimental station, Risano, province of Udine. C. Latium, Istituto per la Patologia Vegetale, Rome; experimental station, Aprilia, province of Latina. D. Emilia, Centro di Fitofarmacia, Dipartimento di protezione e Valorizzazione Agroalimentare, University of Bologne; experimental station, Granarolo Emilia, province of Bologne.

These groups had previously decided on the experimental and analytical methodology. In particular, the following general criteria were agreed upon: (1) vineyard of not less then 3000 m^2; (2) use of Vinclozolin (Ronilan, BASF) at the normal dose recommended for control of *Botrytis* on grapes and, where possible, at double dose; (3) spraying of rows or lots of not less than 20 plants, taking care to avoid border effects and leaving space between test lots; (4) performing each assay at less in triplicate; (5) spraying at the closing of bunches and at the varaison (outset of the ripening phoenomena) stages and 21 days before harvest; (6) recording of salient meteorological data; (7) residue analysis on leaves after each spraying as far as possible at the following intervals: 2 h and 1, 2, 5, 12, and 21 days, but no sampling during rainfall; (8) one assay on grapes 21 days after the final spraying to verify the compliance with the Italian tolerance level (<1.5 μg/g fresh weight on fruits and vegetables 21 days after the last treatment).

In Table I the experimental conditions at each station are set out and in Table II the salient meteorological data for the period June/Sept 1983.

For residue determination and analysis the four laboratories agreed as follows: (1) determination by gas chromatography with packed or capillary columns and EC ^{63}Ni detector; (2) extraction of not less than 5 g of vegetable

Table III. Analysis Methods Adopted in the Various Laboratories

	laboratory			
	A	B	C	D
extraction				
sz of sample	leaves 25 g; grapes 50 g	leaves 25 g; grapes 25 g	leaves 25 g; grapes 50 g	leaves 6 g; grapes 50 g
type of extrctn	leaves homog in acetone; grapes maceration in hexane	leaves maceration in benzene; grapes homog in benzene	leaves maceration in pet. ether; grapes homog in pet. ether (40-60 °C)	leaves homog in acetone; grapes homog in acetone
cleanup	col chromat, (activ Florisil), eluent/ethyl ether (30%) in hexane	col chromat (silica SEP-PAC/cartridges), eluent benzene	sweep codistilln, eluent ethyl acetate	liq partition water and hexane-methylene chloride (1:1, v/v)
anal.				
type	GC glass col packed with SE 30 3% on Chromosorb W 60-80 mesh	GC glass capillary col OV1 film thickness 0.1-0.15 μm	GC glass col packed with QF 5% on Chromosorb WHP 80-100 mesh	GC glass col packed with OV17 1.5% + OV210 1.95% on Varaport 30-80-100 mesh
column temp, °C	210	200	220	170
detector (temp, °C)	EC ^{63}Ni (250)	EC ^{63}Ni (250)	EC ^{63}Ni (275)	EC ^{63}Ni (320)
injector	270 °C	split splitess 3:5	250	250
carrier gas (flow, mL/min)	N_2 (45)	He (4)	N_2 (40)	He (35)
method	ext std	ext std	ext std	ext std
instrum	C. Erba 2400	C. Erba 4200	C. Erba 2400 T	HP J750
detectn lim, ng	0.02	0.02	0.05	0.02
mean recovery, % (SE)	98 (4.2)	93 (2.1)	97 (3.0)	82 (2.5)

[a] Zanini et al., 1980. [b] Barbina Taccheo et al., 1978. [c] Molinari et al., 1978. [d] Flori et al., 1982.

Table IV. Results Obtained at Each Experimental Station (Mean Values of the Residue at Indicated Times following Spraying and Values Normalized to the Residue 2 h after Spraying) (I, Normal Dose; Residue Expressed in μg/g of Fresh Weight)

		vinclozolin on leaves						
		1st spraying		2nd spraying		3rd spraying		
exptl station	days	mean	norm.	mean	norm.	mean	norm.	vinclozolin on grapes: mean
A	0.08	64.0	1.00	120.0	1.00	125.0	1.00	0.40
	1	51.0	0.80	107.0	0.89	113.0	0.90	
	2	37.0	0.58	94.2	0.78	93.3	0.75	
	5	22.0	0.34	58.9	0.49	77.0	0.62	
	12	8.5	0.13	7.5	0.06	20.7	0.16	0.32
	13			5.9	0.05			
	21	2.5	0.04			2.8	0.02	0.31
B	0.08	70.9	1.00	75.8	1.00	136.8	1.00	
	1	55.4	0.78	56.2	0.74	109.5	0.80	
	2	50.6	0.71	42.1	0.55	76.5	0.56	
	4	33.1	0.47			26.9	0.20	
	7	19.6	0.28					
	11					4.0	0.03	
	12			1.4	0.02			
	21					0.3	0.002	0.30
	22	1.0	0.01	0.06	0.001			
C	0.08	77.7	1.00	201.1	1.00	64.8	1.00	0.94
	1	60.0	0.77	160.7	0.80	48.4	0.75	
	2	55.6	0.71	88.9	0.44			
	5	30.4	0.39	40.1	0.20	12.3	0.20	
	12	8.8	0.11			3.8	0.06	0.64
	14			11.1	0.06			
	22	0.7	0.01	4.0	0.02			
	26					0.1	0.002	0.11
D	0.08	216.0	1.00	200.4	1.00	238.9	1.00	
	1	183.0	0.84	53.5	0.27			
	2	55.5	0.26	7.2	0.04			
	5					36.1	0.15	
	12			2.8	0.01	8.5	0.04	1.20
	21	0.2	0.001			5.9	0.02	0.70

sample through homogenization or maceration in nonpolar solvents; (3) cleanup of extracts by chromatography on activated Florisil or Sweep Codistillation columns on Sep-pac cartridge; (4) use of solvents and reagents at maximum grade of purity for the analysis of residues; (5) use of analytical standard 99% Vinclozolin (S.n.I. Ehrenstorfer, D-8900 Augsburg, D.F.R.); (6) preliminary recovery trials of vine leaves fortified at three levels of active principle (0.1, 1.0, and 10.0 μg/g of fresh weight) each repeated 10 times.

In Table III the analytical conditions and instruments of the four laboratories are set out, together with the average performance in each recovery trial.

RESULTS

Tables IV and V give the results obtained at each ex-

Table V. Results Obtained at Each Experimental Station (Mean Values of the Residue at Indicated Times following Spraying and Values Normalized to the Residue 2 h after Spraying) (II, Double Dose; Residue Expressed in μg/g of Fresh Weight)

		vinclozolin on leaves						
		1st spraying		2nd spraying		3rd spraying		
exptl station	days	mean	norm.	mean	norm.	mean	norm.	vinclozolin on grapes: mean
A	0.08	285.0	1.00	141.9	1.00	200.0	1.00	1.24
	1	202.1	0.71	132.9	0.94	174.0	0.87	
	2	148.8	0.52	105.0	0.74	113.0	0.56	
	5	75.0	0.26	74.1	0.52	93.9	0.47	
	12	70.9	0.25	13.0	0.09	42.9	0.21	0.81
	13			5.2	0.04			
	21	33.0	0.12			4.6	0.02	0.50
B	0.08	207.7	1.00	140.5	1.00	160.6	1.00	
	1	174.3	0.84	118.6	0.84	156.9	0.98	
	2	122.1	0.59	70.8	0.50	93.5	0.58	
	4	77.4	0.37			49.7	0.31	
	5			4.7	0.03			
	7	56.0	0.27					
	11					9.2	0.06	
	12			1.7	0.01			
	22	1.5	0.007	0.2	0.001	0.6	0.004	0.60
C	0.08	164.0	1.00	247.8	1.00	142.7	1.00	2.36
	1	106.0	0.64	170.8	0.69	99.5	0.70	
	4			118.7	0.48			
	5					28.3	0.20	1.25
	12	15.2	0.09			13.1	0.09	
	14			21.2	0.08			
	22	2.8	0.02	5.3	0.02			
	26					0.6	0.004	0.66
D		trial not performed						

Table VI. Degradation Curve of Residual Values Normalized to the Initial Value (2 h after Each Spraying, Normal Dose)[a]

station	spraying	A	k	r	r^2	SE	$x_{y/2}$
A	1st	0.86	0.15	0.994	0.988	0.15	3.6
	2nd	1.20	0.24	0.992	0.985	0.19	3.6
	3rd	1.18	0.18	0.991	0.982	0.22	4.6
B	1st	1.01	0.19	0.999	0.999	0.05	3.7
	2nd	1.00	0.32	0.999	0.998	0.13	2.2
	3rd	1.00	0.33	0.992	0.985	0.20	2.1
C	1st	1.07	0.21	0.996	0.993	0.16	3.6
	2nd	0.85	0.23	0.980	0.961	0.26	2.2
	3rd	0.89	0.23	0.997	0.994	0.21	2.4
D	1st	0.85	0.32	0.993	0.986	0.45	1.6
	2nd	0.78	0.37	0.991	0.983	0.43	1.2
	3rd	0.82	0.26	0.979	0.959	0.46	1.8

[a] The parameters indicated refer to the exponential model $y = Ae^{-Kx}$ in which y = normalized residue and x = time in days after spraying. Also shown are correlation coefficients r and determination coefficients r^2, standard error of estimate, and theoretical half-life ($x_{y/2}$).

Table VII. Degradation Curve of Residual Values Normalized to the Initial Value (2 h after Each Spraying, Double Dose)[a]

station	spraying	A	k	r	r^2	SE	$x_{y/2}$
A	1st	0.79	0.09	0.980	0.960	0.19	4.9
	2nd	1.22	0.24	0.983	0.966	0.28	3.8
	3rd	1.04	0.17	0.980	0.961	0.32	4.2
B	1st	1.02	0.22	0.997	0.995	0.14	3.2
	2nd	0.95	0.33	0.992	0.985	0.43	2.0
	3rd	1.02	0.25	0.998	0.996	0.14	2.8
C	1st	0.85	0.17	0.996	0.992	0.19	3.0
	2nd	0.93	0.17	0.998	0.997	0.09	3.5
	3rd	0.84	0.20	0.993	0.966	0.29	2.5
D		trial not performed					

[a] The parameters indicated refer to the exponential model $y = Ae^{-Kx}$ in which y = normalized residue and x = time in days after spraying. Also shown are correlation coefficients r and determination coefficents r^2, standard error of estimate, and theoretical half-life ($x_{y/2}$).

Table VIII. Variance Analysis of Half-Life of Initial Residue at Each Experimental Station, for Each of the Spraying Periods and at Both Doses [E = Experimental Station; S = Spraying Period; D = Dose (Emilia Excluded)]

source	sum of squares	DF	variance	F ratio	F prob
main effects	9.574	5	1.915	3.937	0.104
E	7.410	2	3.705	7.630	0.043
S	1.963	2	0.982	2.022	0.247
D	0.201	1	0.201	0.413	0.555
interactions	1.489	8	0.186	0.383	0.884
ES	1.267	4	0.317	0.652	0.656
SD	0.114	2	0.057	0.118	0.892
ED	0.108	2	0.054	0.111	0.898
explained	11.063	13	0.851	1.752	0.311
residual	1.942	4	0.486		
total	13.005	17	0.765		

Table IX. Variance Analysis of Half-Life of Initial Residue Considering Only the Principal Causes of Variation and Not the Interactions [E = Experimental Station; S = Spraying Period; D = Dose (Emilia Experimental Station Included)]

source	sum of squares	DF	variance	F ratio	F prob
main effects	16.795	6	2.799	10.640	0.000
E	14.696	3	4.899	18.622	0.000
S	1.898	2	0.949	3.608	0.054
d	0.201	1	0.201	0.762	0.397
explained	16.795	6	2.799	10.640	0.000
residual	3.683	14	0.263		
total	20.478	20			

perimental station (average of repeats of each trial), together with the same values normalized against the initial residual datum (2 h after spraying).

The CURFIT program (Spain, 1982) for the analysis of the two-variable model was run on P.C. Apple II with the normalized data: in this preliminary study the decay was considered exclusively as a function of time under assumption that this was a pseudo-first-order process.

Table X. Multiple-Range Test (Significant Minimum Differences) on the Half-Life of Initial Residue against the Experimental Station

Description

gp	no. of cases	mean	SD	SE	min	max	conf limit (95%)
station A	6	4.1	0.54	0.22	3.6	4.9	3.5–4.7
station B	6	2.7	0.69	0.28	2.0	3.7	1.9–3.4
station C	6	2.9	0.59	0.24	2.2	3.6	2.2–3.5
station D	3	1.5	0.30	0.18	1.2	1.8	0.8–2.3
total	21	3.0	1.01	0.22	1.2	4.9	2.5–3.4

Homogeneity of Variance Test

Cochrans C = 0.388; P = 0.698
Bartlett-Box F = 0.439; P = 0.725
variance max/variance min = 5.043

Analysis of Variance

source	sum of squares	DF	variance	F ratio	F prob
station	14.696	3	4.899	14.40	0.0001
error	5.782	17	0.340		
total	20.478	20			

Pairs of Groups Significantly Different at the 0.050 Level

	station			
	A	B	C	D
A		*	*	*
B	*			*
C	*			*
D	*	*	*	

The best bivariate fitting of experimental data was the classical exponential decay model

$$Y_x = Y'_x/Y_i = Ae^{-KX} \quad (1)$$

where Y'_x = mean residue at time X and Y_i = mean residue initially. A defines the residue at $X = 0$, and K is a rate constant.

With this model the theoretical half-life of the initial residues is easily obtained by putting in (1) $Y = 0.5Y_i$

$$\log \tfrac{1}{2} = \log A - KX_{0.5y} \quad (2)$$

that is

$$X_{0.5y} = \log (2A)/K \quad (3)$$

The exponential equations obtained in the various experimental stations for successive treatments are in Tables VI, and VII; the correlation coefficients r, the determination coefficients r^2, and the standard error of the estimate are also given.

CONCLUSIONS

It was immediately evident that residue decay at the four stations was affected by experimental conditions at each station: this can be seen from the theoretical half-life of the original residue, which ranges from a minimum of 1.2 days to a maximum of 4.6 days after spraying with normal dose and from 2.0 to 4.9 days with double dose. The fastest decay was in Emilia on a red grape (Sangiovese): this may be due to the greater daily temperature range and longer hours of sunshine at this station, and further research on the climate influence is then suggested.

In each vineyard the behavior of the residue varied after successive sprayings perhaps due to seasonal changes, but the variation from station to station was greater.

The analysis of variance (Tables VIII and IX) attaches the highest significance to the variations caused by the differential location of the stations, whereas those caused by dose or by successive sprayings are not sufficiently pronounced to be significant. Interaction between the cause of variation under consideration was not significant. A multiple-range test (least significant difference between the experimental stations) showed that the Piedmont station gave initial residue half-lives significantly higher than other stations taken together (Table X) and Emilia gave the lowest values.

Table XI. Mean Ratio between Residues from Double and from Single Doses

	station				
spraying	A	B	C	mean	no. of cases
1st	6.18	2.54	2.40	3.96	15
2nd	1.22	2.07	1.38	1.58	16
3rd	1.53	1.65	3.18	2.06	17
mean	2.98	2.06	2.38	2.49	48
no. of cases	18	17	13		

Analysis of Variance

source	sum of squares	DF	variance	F ratio	F prob
mean effects	55.290	4	13.822	5.614	0.001
station	6.320	2	3.150	1.283	0.289
spraying	47.702	2	23.851	9.687	0.000
interaction ed	54.481	4	13.620	5.532	0.001
explained	109.771	8	13.721	5.573	0.000
residual	96.026	39	2.462		
total	205.797	47	4.379		

The mean ratio between the residue from double and normal dose was 2.49; the analysis of variance of this value in relation to the station and spraying period (Table XI) showed significant differences by period, but not by experimental station, although the interaction had high significance.

Experimental results show that degradation of Vinclozolin on leaves was fairly rapid in the experimental stations on the plain (Emilia, Latium); the more sheltered position of the Piedmontese hills resulted in longer half-life times.

The exponential model was close to actual decay in all the experimental stations and at both doses. The half-life of the residue of the active principle after spraying was independent of dose, but dependent on the station. From a superficial examination the effect of climatic variations would not appear to be relevant, since the degradation rate did not vary notably with the spraying period. Much more probably this complex of factors influenced the residual

level remaining from the two doses at each station: the high significant interaction between the experimental station and the spraying period on the variation of the double/single dose ratio suggests that the amount of pesticide acts on the residue level differently according to the environmental and experimental conditions.

In no case was the residue on the grapes above the Italian legal limit, not even as a consequence of excessive doses.

The experiments carried out appear to have confirmed the general hypothesis that the degradation of pesticides on the plant is a phenomenon that varies with the environment, rather than the dose: the locality and period of spraying are factors that must definitely be taken into account when establishing spraying period and suitable intervals between final spraying and harvest.

Registry No. Vinclozolin, 50471-44-8.

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Mouse Monoclonal Antibodies against Paraoxon: Potential Reagents for Immunoassay with Constant Immunochemical Characteristics

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Mice were immunized with (*p*-aminophenyl)paraoxon conjugated to protein by diazotization. The production and isolation of permanent hybridoma cell lines from the splenic lymphocytes of the immunized animals are described. The specificity of the two resultant paraoxon-specific monoclonal antibodies was tested with paraoxon analogues and seven insecticides. Significant cross reactivity was found with parathion and (*p*-aminophenyl)paraoxon, while no interfering compounds were found among the other insecticides tested. The affinities of the monoclonal antibodies were determined to be 9.4×10^4 and 1.7×10^5 L/mol. Specificity determinations proved the antibodies to be chemically unique. When incorporated into a competitive inhibition enzyme immunoassay, the antibodies provided quantification of paraoxon that compared favorably with gas chromatography on the basis of precision and specificity. The better of the two antibodies produced a response linear from 10 to 100 μg/mL with a minimum detectable concentration of 1 μg/mL in an assay requiring 50 μL of sample. The direction of ongoing work to improve sensitivity is discussed.

INTRODUCTION

Present methods of analysis for insecticides in environmental samples require time-consuming isolation and cleanup procedures, expensive analytical instruments, and highly trained personnel (Association of Official Analytical Chemists, 1980; U.S. EPA, 1980). Radioimmunoassays (Ercegovitch et al., 1981; Langone and Van Vunakis, 1975; Wing and Hammock, 1979) provide the sensitivity and specificity of immunoassay and reduce the need for extensive cleanup steps. However, they also involve expensive equipment and highly trained personnel plus radiation risk and long analysis times.

Competitive inhibition enzyme immunoassay (CIEIA) is a rapid, simple, and inexpensive alternative. Work in our laboratory using heteroantisera has shown the feasibility of this technique applied to paraoxon (Hunter and Lenz, 1982) and soman (Hunter et al., 1983). A similar assay has been successfully applied to parathion residues (Al-Rubae, 1978). An informative review has been published by Hammock and Mumma (1980).

Hapten-specific polyclonal antisera, usually raised in rabbits, form the basis for the immunoassays discussed above. As analytical reagents, heteroantisera posses two substantial drawbacks. They are subject to the variation in immunoglobulin composition and specificity that arise during the maturation of the in vivo immune response (Eisen, 1980). Further, a given heteroantiserum remains available only over the finite lifespan of the immunized animal.

We undertook the present work to supplant the use of heteroantisera in the paraoxon immunoassay procedure. This was accomplished by the development of mouse hybridoma cell lines that produce paraoxon-specific mono-

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