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QUANTITATIVE CALCULATION OF GAS CHROMATOGRAPHIC PEAKS IN PESTICIDE
RESIDUE ANALYSES

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Quantitative Calculation of Gas Chromatographic Peaks in Pesticide Residue Analyses

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Comparison of five methods for calculating gas chromatographic peaks (disc integration, triangulation, peak height $\times$ width at half height, $R_t \times$ peak height, and peak height) shows no significant difference in the calculated results when aldrin, heptachlor epoxide, and dieldrin are injected into a gas chromatograph equipped with electron capture detector.

Problems are associated with the calculation of toxaphene, chlordane, DDT, and BHC. The author makes suggestions for calculating these four residues when present individually and in combinations with other pesticides. Use of the last four toxaphene peaks compares favorably with results obtained by using the entire toxaphene curve. A method for calculating DDT in the presence of toxaphene, based on the construction of "parallel lines", gives 89–100% of the DDT in the presence of 1–10 parts of toxaphene. Results for chlordane obtained by adding heights of individual chlordane peaks are shown to be close to results obtained with an integrator. When electron capture detection is used for determining the α , β , γ , and δ -isomers of BHC, the response of the β -isomer is found to be approximately 50% less than response of the other isomers, thus pointing up the need for use of individual BHC isomers for electron capture gas-liquid chromatography quantitation.

Major advances were made in the field of pesticide residue analyses with the advent of the gas chromatograph. Detection of single and multiple residues at the nanogram and picogram level and calculation of these pesticides as parts per million and parts per billion are routine in pesticide residue laboratories today.

Methods of Quantitating Peaks

Many methods of quantitating gas chromatographic peaks have been presented in the literature, and the choice of one method over another depends mainly on the type of

peaks to be measured and the operator's preference. Basically, the quantitative interpretation of a gas chromatogram is made on either peak height or peak area. Methods listed in the literature include the following:

(a) *Peak height*.—To quantitate by the peak height method, the baseline is first constructed beneath the peak, the zone center of the peak is located, and a vertical line is dropped from the zone center to the base. Peak height measurements are usually recommended for early eluting peaks and for very small peaks. If standard and unknown peaks are approximately the same size, peak height method is less subject to operator error in measuring than triangulation or width at half height for very narrow peaks. Several authors (1–4) have stated that peak height is probably the fastest method for quantitating a peak but not the most reliable. Peak height is very sensitive to changes in operating conditions. If quantitation is to be achieved by this method (or any other method), operating parameters should be closely controlled.

(b) *Product of peak height and width of the peak at half height*.—This method has been described by several workers (5, 6). Peaks should be symmetrical or nearly so for reasonable accuracy. The method is limited by the accuracy with which the peak height of narrow peaks can be measured. Hawkes and Russell (7) have shown that peak width at half height for a peak with sloping baseline is the line D'E" in Fig. 1.

(c) *"Area" as a product of retention time and peak height*.—A method of determining peak "area" as a product of retention time and peak height has been presented by Bartlet and Smith (8, 9). Iverson (10) compared this method with the triangulation method and concluded that "there was no statistically demonstrable difference in the precision of the two methods." The author prefers this method or the peak height

Fig. 1—Peak parameters for peaks on flat baseline (I) and sloping baseline (II) by Methods for Quantitating Peaks (a)–(g); see text.

Peak I	Peak Parameters	Peak II
FG	Baseline	F'M'
A	Zone center of peak	A'
AB	Peak height	A'B'
AC	Peak half-height	A'C'
DE	Width at half-height	D'E''
FG	Peak elution width	F'G'
KJ+LJ	Inflection tangents	K'J'+L'J'
KL	Base (triangulated peak)	J'K'
JB	Height (triangulated peak)	L'N'
IA	Retention volume	I'A'
OA	Apparent retention time	O'A'

method to quantitate overlapping peaks where the amount of overlap does not obscure the individual peak heights (Figs. 2b and 2c). Bartlet and Smith (8) have evaluated the mutual effects of overlapping peaks and the possibility of obtaining corrected peak heights where overlap obscures the true peak height.

(d) *Cutting out and weighing the peaks.*—This method, in which the peak area is determined by cutting out the peaks and weighing the paper on an analytical balance, is not often used since it is time consuming. Dal Nogare and Juvet (5) and Dimbat, *et al.* (3) point out that its accuracy depends upon the uniformity and moisture content of the chart paper and the care exercised by the operator in cutting the paper. As a method of calculation it should not be overlooked when the integrator is not functioning properly and chromatograms of complex pesticides such as toxaphene must be quantitated.

(e) *A mechanical or electrical integrator attached to the recorder.*—The integrator method is claimed by some authors (3, 11) to be a most convenient and accurate method of quantitating area. It loses simplicity, however, whenever baseline corrections need to be made. In our laboratory, disc integrators are calibrated to obtain the number of disc units (DU) per square inch (or arbitrary distance). Our baseline correction value is obtained by taking the area of the trapezoid ($A = \frac{a+b}{2} \cdot h$) under the peak and multiplying by the DU calibration factor. The method is used extensively for residue calculations and is the method of choice for toxaphene, Strobane®, chlordane, and other pesticides which elute over a long period of time.

(f) *Triangulation.*—The baseline and inflectional tangents to the peak form a triangle for which $\text{Area} = \frac{1}{2} \text{base} \times \text{altitude}$. This method requires extreme care in construction of the inflectional tangents and care in measurement. It is subject to error when triangulation of very narrow peaks is attempted. Peaks on sloping baselines present problems which operators deal

with in various ways. For example, for the triangle J'K'L' in Fig. 1, the author will calculate area, $A = \frac{1}{2}(J'K')(L'N')$. Another operator will bisect K'L' at point P' and rotate the line K'L' until it is perpendicular to the bisector, thus forming the triangle base.

(g) *Planimetry.*—The use of a planimeter is one of the standard methods of calculating peak area but is not used to any extent in the FDA District laboratories.

Peak parameters used in methods (a) through (f) are illustrated in Fig. 1. Baseline construction, preferred by the author, for some typical pesticide gas chromatographic peaks is illustrated in Fig. 2.

Applications

Five of the above methods for quantitating peaks were compared for eight injections of aldrin, heptachlor epoxide, and dieldrin into a gas chromatograph equipped with a tritium electron capture detector (12).

Methods compared were disc integration, triangulation, peak height $\times$ width at half height, $R_t \times$ peak height, and peak height. Results are summarized in Table 1. No significant difference was found in the five methods used for quantitating these peaks. The only method for which the calculated quantities were usually higher than the injected quantities was the disc integrator method.

Pesticides which are mixtures of isomers present problems in calculation. When they are found together, e.g., toxaphene and DDT, the problem of quantitation becomes even more difficult. In the following sections the author offers suggestions for handling toxaphene, chlordane, DDT, and BHC, based on work done in her laboratory. A column of 10% DC-200¹ stationary phase on a support of 90–100 mesh Anakrom ABS² was used.

Calculation of Toxaphene

Quantitative calculation of toxaphene or Strobane® is difficult, but reasonable accuracy can be obtained to 2 significant figures. To

¹ DC-200—Dow Corning silicone oil (12,500 cst).

² Anakrom ABS—This product of Analabs, Inc. (9 Hobson Ave., Hamden, Conn. 06518) is a flux-calcined diatomaceous earth aggregate.

Table 1. Comparison of quantities of aldrin, heptachlor epoxide, and dieldrin found by 5 methods of calculation

	Injected		Peak Height, % Full Scale	Disc Integrator	Triangulation	Peak ht $\times$ Width at Half Ht	$R_t \times$ Peak Height	Peak Ht
	μ l	ng		ng	ng	ng	ng	ng
Aldrin	1.1	0.55	6.5	0.47	0.46	0.45	0.45	0.44
	2.0	1.00		1.00	0.98	0.96	0.95	0.96
	3.0	1.50		1.57	1.49	1.45	1.44	1.44
	3.9 ^a	1.95		—	—	—	—	—
	4.9	2.45	TO	2.55	2.42	2.42	2.42	2.42
	5.9	2.95		3.16	2.88	2.91	2.93	2.90
	6.9	3.45		3.42	3.12	3.26	3.28	3.25
	7.8	3.90	56	4.09	3.62	3.77	3.80	3.77
Heptachlor epoxide	1.1	0.55	4.5	0.47	0.47	0.49	0.44	0.44
	2.0	1.00		1.03	0.97	0.98	0.98	0.98
	3.0	1.50		1.52	1.43	1.44	1.43	1.44
	3.9 ^a	1.95		—	—	—	—	—
	4.9	2.45	TO	2.46	2.39	2.42	2.44	2.42
	5.9	2.95		2.94	3.00	2.90	2.91	2.89
	6.9	3.45		3.42	3.38	3.24	3.26	3.24
	7.8	3.90	42	3.76	3.70	3.70	3.72	3.69
Dieldrin	1.1	0.55	3.5	0.50	0.49	0.52	0.46	0.46
	2.0	1.00		0.94	0.91	0.96	0.91	0.91
	3.0	1.50		1.46	1.39	1.47	1.39	1.39
	3.9 ^a	1.95		—	—	—	—	—
	4.9	2.45	TO	2.53	2.51	2.41	2.40	2.40
	5.9	2.95		3.08	2.99	2.99	3.01	2.98
	6.9	3.45		3.30	3.12	3.16	3.29	3.26
	7.8	3.90	31	4.03	3.94	3.90	3.86	3.82

^a 3.9 (1.95 ng) used as basis for calculation.

calculate toxaphene on electron capture gas chromatographs, we (a) adjust sample size so that toxaphene major peaks are 1-3 inches from baseline; (b) inject a toxaphene standard that is estimated to be within ± 10 nanograms of the sample; (c) construct the baseline of standard toxaphene between its extremities; and (d) construct the baseline under the sample, using the distances of the peak troughs to baseline on the standard as a guide (Figs. 3, 4, and 5). This procedure is made difficult by the fact that the relative heights and widths of the peaks in the sample will not be identical to the standard. A pure toxaphene standard that has been passed through a Florisil column will show a shorter retention time for peak X and an enlargement of peak Y. If DDT is present, it will superimpose itself on peak V. To determine the approximate base-

line of the DDT, we draw a line connecting the trough of peaks U and V with the trough of peaks W and X and construct another line parallel to this line which will just cut the top of peak W (Fig. 2-1). This procedure was tested with various ratios of standard toxaphene-DDT mixtures, and the results of added and calculated DDT and toxaphene by the "parallel lines" method of baseline construction are summarized in Table 2.

Tables 3a and 3b compare results obtained by calculating total toxaphene and its last 4 peaks. In the author's opinion the results justify the use of the latter method for calculating toxaphene in a sample.

The baseline for methoxychlor superimposed on toxaphene (Fig. 5b) was constructed by overlaying the sample on a toxaphene standard of approximately the same concentra-

Fig. 2—Baseline construction for some typical gas chromatographic peaks. a, symmetrical separated flat baseline; b and c, overlapping flat baseline; d, separated (pen does not return to baseline between peaks); e, separated sloping baseline; f, separated (pen goes below baseline between peaks); g, α - and γ -BHC sloping baseline; h, α -, β -, and γ -BHC sloping baseline; i, chlordane flat baseline; j, heptachlor and heptachlor epoxide superimposed on chlordane; k, chair-shaped peaks, unsymmetrical peak; l, *p,p'*-DDT superimposed on toxaphene.

tion (Fig. 5a) and viewing the charts against a lighted background.

Calculation of Chlordane

Chlordane is composed of several compounds including heptachlor, chlordene, and α - and β -chlordane. Fig. 4a shows standard chlordane.

We have found chlordane present in a variety of samples but have never found it where the peaks were present in the same ratio as those for the standard. The A and B peaks (Fig. 4b) appear to weather more rapidly than the other peaks. Fig. 4b shows chlordane in a sample of rice bran. An additional peak between peaks D and E with retention time of heptachlor epoxide has been found for some samples.

Calculation of chlordane in a sample should include all peaks. A calculation based on α - and β -chlordane only may be (a) high if the α - and β -peaks from a chlordane mixture are used as standard, since ratio of all other peaks is not the same, or (b) low if pure α - and β -chlordane isomers are used as standard

Table 2. % DDT and toxaphene calculated by "parallel lines method" for various ratios of toxaphene-DDT mixtures^a

Ratio DDT:Tox.	DDT			Toxaphene		
	Added, ppm	Found, ppm	%	Added, ppm	Found, ppm	%
1:1	8.2	7.8	95	8.2	a	
1:1	21	b	—	21	23	109
2:1	40	b	—	20	22	110
1:2.5	7.8	7.8	100	20	21	105
1:5	8.0	7.7	96	40	43	108
1:7.5	8.0	7.7	96	60	63	105
1:10	8.0	7.1	89	80	82	102

^a a—Peaks too small to calculate.

b—Peaks too large to calculate.

and the other chlordane peaks in the sample are neglected. We calculate chlordane residues by using the total area of peaks A, B, C, D, E, and F. Peak G may be obscured in a sample by the presence of other pesticides. If G is not obscured, it is calculated for both standard and sample. If heptachlor epoxide is present as a small peak, it is calculated as

Fig. 3a—Baseline construction for multiple residues with standard toxaphene.

Fig. 3b—Baseline construction for multiple residues with toxaphene, DDE and *o,p'*-, and *p,p'*-DDT.

total chlordane. If the heptachlor and heptachlor epoxide are much out of proportion as in Fig. 2j, we calculate these separately and subtract their areas from total integrator area to give a corrected chlordane area. As in the preceding section on toxaphene, we first inject a standard in which the α - and β -isomers are estimated to be approximately the same size as the α - and β -isomers in the sample. The baseline is constructed beneath the standard from the beginning of peak A to the end of peak F as shown in Fig. 4a. The distance from the trough between peaks E and F to the baseline is used to construct the baseline under the sample. Fig. 4b shows how the presence of toxaphene caused the baseline under chlordane to take an upward angle. When the size of the α - and β -isomers in standard and sample are the same, the distance from the trough of the isomers (peaks E and F) to the baselines should be the same.

Calculation of chlordane area is usually done with an integrator. Table 4 shows a comparison of the integrator method and the addition of peak heights method for several samples containing chlordane. The peak heights of peaks A, B, C, D, E, and F were measured in millimeters from peak maximum of each to the baseline constructed under the total chlordane area and were then added together. These results are too close to ignore this method of "peak height addition" as a means of calculating chlordane. We hesitate to recommend it, however, because of the fact that not all the peaks are symmetrical or are present in the same ratio in standard and in sample. This method does offer a means of calculating results if the integrator should fail.

Calculation of DDT

DDT found in samples most often contains both *o,p'*- and *p,p'*-DDT. The ratio of *o,p'*

Table 3a. Toxaphene, ppm, calculated in presence of DDT in alfalfa hay by various methods of calculation

Disc Integrator Method		
Whole Curve, ^a ppm	Last 4 Peaks, ^b ppm	Cut and Weigh Method, ^c ppm
24	24	23
72	63	71
32	37	41
30	27	31
102	102	70
110	112	96
124	130	130

^a Total toxaphene corrected for DDT-DDE present. Sample peaks compared to toxaphene standard which closely matched apparent toxaphene present.

^b Peaks after DDT has eluted.

^c Estimated toxaphene curve under DDT-DDE peaks.

Table 3b. Calculation of added toxaphene by the disc integration of total curve and last 4 peaks

Product	Added, ppm	Found, ppm	
		Whole Curve	Last 4 Peaks
Raw milk	2.5	2.0	2.4
Raw milk	5.0	4.5	4.4
Dried corn	0.50	0.42	0.43
Dried corn	1.0	0.78	0.79

Table 4. Chlordane, ppm, in samples by integrator and peak height methods

	Integrator, ppm	Peak Height, ppm
Cottonseed meal	1.58	1.47
Cottonseed hulls	0.047	0.045
Cottonseed hulls	0.40	0.40
Cottonseed hulls	0.41	0.42
Rice bran	0.11	0.11
Rice bran	0.12	0.12
Rice bran	0.30	0.27
Rice bran	0.34	0.30
Rice polish	0.63	0.57
Rice bran	0.55	0.45
Rice bran	0.70	0.50
Brown rice	2.19	1.99

to p,p' is not necessarily the same in samples as it is in standards. We have found potatoes receiving a post-harvest treatment of DDT and stored with a blanket of DDT dust to have an o,p' peak equivalent to 40–50% of total o,p' and p,p' combined.

Dairy products containing DDT also contain DDE and DDD. If DDD is present in any

appreciable quantity it can obscure the o,p' peak on some GLC columns. For routine work we recommend calculation of p,p' -DDT on the basis of pure p,p' standard. When o,p' -DDT and DDD are not clearly separated, they are calculated as total DDD and reported as DDD. A synthetic standard containing known quantities of o,p' and p,p' isomers is used for calculating total DDT. Peaks are calculated as ppm o,p' and p,p' , added, and reported as total DDT.

Calculation of Benzene Hexachloride

Technical grade benzene hexachloride is a cream-colored amorphous solid with a very characteristic musty odor; it consists of a mixture of six chemically distinct isomers and one or more heptachlorocyclohexanes and octachlorocyclohexanes (13).

Commercial BHC preparations may show a wide variance in the percentage of individual isomers present. The elimination rate of the isomers fed to rats was 3 weeks for the α , γ , and δ -isomers and 14 weeks for the β -isomer (14). Thus it may be possible to have any combination percentage of the various isomers in raw agricultural and dairy products. BHC found in dairy products in this laboratory usually has a large percentage of β -isomer.

Individual isomers (α , β , γ , and δ) were injected into gas chromatographs equipped with flame ionization, microcoulometric, and electron capture detectors. Table 5 gives a summary of response obtained for the individual isomers with these detectors. Response for the four isomers is very nearly the same whether flame ionization or microcoulometric GLC is used. The α , γ , and δ -isomers show equal electron affinity. β -BHC shows a much weaker electron affinity compared to the other isomers.

When β -BHC is found in a sample by electron capture, it should be calculated separately (by peak height method or by peak height $\times$ retention time) from a reference standard of β -isomer. Individual isomers are then added and reported as total BHC.

If γ -isomer (lindane) only is present in a product, it should be calculated and reported as lindane with γ -isomer as a reference standard. When isomers other than the γ -isomer are present, we cannot draw a common rule, i.e., calculate γ as part of total BHC. If, however, the amount of γ -isomer is not greatly out of proportion to the other isomers present, the residue is calculated as total BHC.

In summary, BHC should be calculated from individual isomer reference standards if

Fig. 4a—Baseline construction for multiple residues: standard chlordane.

Fig. 4b—Baseline construction for multiple residues: rice bran with chlordane, toxaphene, and DDT.

electron capture gas chromatography is used. Total BHC in products can be determined by total area of all isomers present from a reference standard of mixed isomers by micro-coulometric gas chromatography.

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Fig. 5a—Baseline construction for multiple residues: standard toxaphene.

Fig. 5b—Baseline construction for multiple residues: rice bran with BHC, toxaphene, DDT, and methoxychlor.

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Table 5. Detector response and retention ratios for the individual isomers of benzene hexachloride

	Flame Ionization ^a			Microcoulometric ^b			Electron Capture ^c		
	Response			Response			Response		
	Peak height $\times R_t$ mm ² /5 μ g	%	Retention Ratio ^d	Area in. ² /1 μ g	%	Retention Ratio ^d	Peak height $\times R_t$ mm ² /1 ng	%	Retention Ratio ^d
α	1990	100 ^e	0.38	3.94	100 ^e	0.48	1560	100 ^e	0.39
β	1890	95	0.41	3.76	95	0.54	590	38	0.43
γ	2070	104	0.46	3.78	96	0.56	1550	99	0.47
δ	2060	104	0.47	3.25	82	0.61	1660	106	0.49

^a Column: 10% DC-200 (12,500 cst) on 90/100 Anakrom ABS, 6' $\times$ 6 mm i.d. Column temp., 200°C; flow rates: 125 ml/min. (N₂), 450 ml/min. (air), 45 ml/min. (H₂); detector temp., 265°C; injection temp., 230°C.

^b Column: 10% DC-200 (12,500 cst) on 80/90 Anakrom ABS, 6' $\times$ 1/4" o.d.

Column temp., 215°C; flow rate, 100 ml/min.; injection temp., 240°C.

^c Column: 10% DC-200 (12,500 cst) on 90/100 Anakrom ABS, 6' $\times$ 6 mm i.d.

Column temp., 202°C; flow rate, 130 ml/min.; detector temp., 207°C; injection temp., 220°C.

^d Relative to aldrin (1.00).

^e % response for α -isomer taken to be 100%.

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