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QUANTITATION OF ENVIRONMENTAL CONTAMINANTS BY FUSED-SILICA CAPILLARY COLUMN GAS CHROMATOGRAPHY–MASS SPECTROMETRY WITH MULTIPLE INTERNAL STANDARDS AND ON-COLUMN INJECTION

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SUMMARY

Data obtained by using an on-column injector, retrofitted to a Finnigan 9500 gas chromatograph, and the original splitless injector are compared. The on-column injector gives a higher precision and improved sample transfer, leading to greater sensitivity and less discrimination. The choice of final sample solvent is made on the basis of the chromatographic requirements. The criteria for the selection of multiple internal standards for environmental sample analysis by gas chromatography–mass spectrometry, gas chromatography–electron-capture detection and high-performance liquid chromatography are discussed.

INTRODUCTION

Over the past 3 years there has been a considerable growth in the number of reports describing the use of capillary columns, particularly fused-silica columns, for the quantitative gas chromatographic–mass spectrometric (GC–MS) determination of contaminants in environmental samples[1](Table I). The use of narrow-bore, high-efficiency columns, with the inherent small (0.1–2μl) injection volumes, and long analysis

TABLE I

SUMMARY OF REF.1, ENVIRONMENTAL SCIENCE SECTION

	Type of column (%)			*Internal standards reported (%)*
	Packed	*Glass capillary*	*Fused-silica*	
Jan–June '80	58	42	0	20
July–Dec '80	77	33	0	19
Jan–June '81	63	37	0	2
July–Dec '81	64	34	2	11
Jan–June '82	59	34	7	5
July–Oct '82	33	31	36	5

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times (>40 min) for multicomponent analyses has made the use of internal standards (ISs) mandatory in any rigorous quantitative determination. While this is reflected in most branches of medical science (in the Clinical Chemistry section of ref. 1, 73 % of the applications used ISs) the same does not appear to be so for environmental science (in ref. 1, 10% of these applications use ISs). Of this 10% nearly half were for the analysis of tetrachlorodibenzo-*p*-dioxins (TCDDs) by use of ^{37}Cl-labelled isomers. Although many applications in the environmental field use GC–MS for qualitative identification of pollutants, it is a serious indictment of much of the published quantitative data that no IS was used in obtaining it.

One of the main problems in this field is the choice of IS. In keeping with medical science some methods have employed isotopic analogues[1,2] particularly for TCDD and polyaromatic hydrocarbon (PAH) determinations, but clearly this cannot be seriously contemplated on financial or logistical grounds for the extensive multi-component analyses of both pollutants and metabolites in the environmental field. Since one of the main sources of error in quantitative capillary GC–MS, apart from the initial sampling, occurs during sample introduction, it is often sufficient to add the IS just prior to injection, after any preparative step[3]. (This would not be true where derivatization or non-quantitative extractions are required in preparation. The IS would then be added at this stage.) If the procedure is adopted, as it has been in this laboratory, then some of the restrictions on the selection of IS are less severe, and do give a wider range of choice of compound.

The criteria for selection were based, firstly, on the fundamental requirements of purity, stability (thermal and photochemical), absence from the sample(s) and availability, preferably at reasonable cost. Secondly, this laboratory uses GC–MS, GC with electron-capture detection (ECD)[4] and high-performance liquid chromatography (HPLC) with ultraviolet (UV)[5] and fluorescence detection, and samples are often cross-checked by a second method. It was highly desirable to use ISs which were detectable with as many of these systems as possible. The ISs were therefore required to (i) have a similar retention time (R_x) but to be separated from their analyte(s), (ii) have a limited number of mass spectral fragments, preferably $m/z > 120$ (above the MS background), (iii) be electron-capturing, or UV-absorbing (and/or fluoresce). Until recently, the introduction of a single IS was sufficient to obtain the necessary degree of precision for packed columns, but in order to improve the precision of data for capillary columns and on-column injection (OCI) it is necessary to incorporate multiple ISs with the samples for quantification of analytes eluted over a wide boiling range. Sauter *et al.*[6] have reported this in their extensive study of the use of fused-silica columns in the GC–MS analysis of priority pollutants. In our work similar principles have been investigated for ISs that may be used in conjunction with other chromatographic techniques in which unlabelled compounds are used. The discrimination experienced in the injection of compounds of differing polarity and volatility has been considerably improved by replacing the splitless injector with an OCI.

EXPERIMENTAL

Materials

Pentane, *n*-hexane and isooctane were supplied as HPLC grade from Rathburn Chemicals (Walkerburn, U.K.). Octane and decane and the bromohydrocarbons were

supplied as Gold Label from Aldrich (Sittingbourne, U.K.). Other chemicals were supplied as follows (see Glossary for nomenclature): dieldrin (Shell Research, Sittingbourne, U.K.) ; Eulan W.A. New (Bayer, Shipley, U.K.).The PAD metabolites and PCSD active ingredients of Eulan W.A. New were synthetized and purified at this laboratory from the Eulan W.A. New formulation[4]. Permethrin and terbutryn metabolites (Ciba Geigy, Manchester, U.K.). The deuterated polyaromatic hydrocarbons were gifts from Dr. R. Law, MAFF, Burnham-on-Crouch, U.K. and the dechlorane from Dr. L. Reutergardh, University of Stockholm, Sweden.

Instrumentation

The instrumentation used was a Finnigan 9500 gas chromatograph fitted with a split/splitless, Grob type, injector, interfaced to a Finnigan 3200F mass spectrometer and 6100 data system. The data handling software for printing multiple chromatograms and the algorithm for quantitative data were written and developed at this laboratory by the authors. The GC column was 25 m × 0.25 mm I.D. fused-silica CP Sil 5 (Chrompack, London, U.K.) and installed from the injector through the interface oven directly into the ion source. The GC programmes are given in the text. The interface temperature was 240°C, and the transfer line 250°C, unless the final column temperature extended beyond 250°C, when the interface was adjusted to 10° above that final value. The MS conditions were: source current, 0.30 μA; electron energy; 25 eV (to avoid ionization of helium); electron multiplier, 1500 V and a preamp setting of either 10^{-7} or 10^{-8}, depending on the concentrations of the sample.

An on-column injector, OCI-2 (SGE, Milton Keynes, U.K.) was retrofitted to the Finnigan 9500 GC oven top, between the end of the interface oven and the GC oven edge by using the manufacturers recommendations. (Note: Following the completion of this work the injector was re-installed at the existing injector location after first removing the injector block, the solid column and Grob split/splitless injector.)

Injection technique

The split/splitless injector was used in the splitless mode with 20 ml/min of helium as the purge gas. The split valve was closed, the sample (1μl) was injected at 250°C and the injector vented after 45 sec, and the programme commenced after 1 min.

The sample was introduced into the on-column injector at ambient temperature, using a syringe with 0.17 mm I.D. fused-silica needle (SGE) through the pneumatic seal. Following insertion and sealing the syringe needle, the carrier gas pressure was permitted to become re-established (10–20 sec) before the sample was injected. The injection time was <1 sec for 1-μl samples and between 2 and 3 sec for 2- to 5-μl samples.

RESULTS AND DISCUSSION

On-column injection

An on-column injector was retrofitted to the Finnigan 9500 to improve the reproducibility of sample introduction of the splitless injector, particularly for relatively less volatile compounds, such as aromatic amines and sulphonamides. Following installation, the system was tested with a wide variety of mixtures and found to be

superior to the splitless system in sensitivity, sample discrimination and reproducibility. Many of these points have been adequately covered by other workers[7,8] so that this report is confined to additional information only.

Grob[8] has reported that up to 8 μl may be injected over a period of 20 sec without harmful effects on the column or to the detriment of the chromatographic analysis particulary for chemically bonded phases. While this holds true for GC alone, the upper limit on solvent volume for GC–MS, where the column is interfaced directly into the ion source, is about 5 μl. (The more efficient turbomolecular pumps on newer instruments will handle a greater solvent volume.) This volume can safely be injected over 2–3 sec with the column temperature at or between 5 and 10° above the boiling point of the solvent. More reproducible results were also obtained when the flexible fused-silica syringe needle was lengthened to 12 cm to extend into the column beyond the injector inside the GC oven upon injection. A summary of results obtained for two more difficult analytes are given in Table II. Terbutryn, a triazine-based aquatic herbicide and poly-chlorinated amino diphenyl ethers (PADs) were both difficult to quantify by use of the splitless injector, but showed a marked improvement when introduced via the OCI. 9-Bromoanthracene (9–BA) was used as the internal standard for the determination of terbutryn and decachlorobiphenyl (DCBP) with the PADs.

TABLE II

COMPARISON OF INJECTION PRECISION WITH SPLITLESS AND ON-COLUMN INJECTORS

RSN = Relative scan number; RPA = relative peak area.

Analyte	*m/z*	*Internal standard*	*m/z*	*No. of analyses*	*Coefficient of variation (2σ)*			
					OCI		*Splitless*	
					RSN	*RPA*	*RSN*	*RPA*
Terbutryn	185	9–BA	256	7	2.3	3.9	1.1	16.6
De-ethyl terbutryn	156		256	7	2.5	5.9	2.6	
PADs	321	DCBP	500	7	1.6	6.1	2.63	20.7
DCBP	498	DCBP	500	7	–	2.0	–	6.8

Solvent choice

Following Grob and Grob's detailed descriptions of the solvent effects immediately following injection[9] most workers have chosen to inject their samples in pentane or *n*-hexane at ambient or just above ambient temperatures. This is particularly suitable for the determination of analytes with a wide boiling range or for volatile compounds. However, many of the pesticide residues and industrial pollutants analysed in this laboratory are less volatile, eluted from most columns at higher (>150°C) temperatures. Therefore, this means that when a volatile solvent, like *n*-hexane, was used, the whole system required cooling from 250–300°C to 70°C or less, injecting and then programming ballistically or at a fast rate, *e.g.*, 20°C/min, to the starting temperature (150–180°C) of the analytical programme. This wasted time in unnecessary cooling,

involved fast, less reproducible temperature programme ramps, and stressed the column by unnecessary cycling.

A successful alternative approach has been to select the starting temperature and programme rate as determined by the separation requirements of the analytes, in an identical way that a packed column program is selected. A solvent of the appropriate boiling range, within 20°C of the initial temperature, is selected and used as the sample solvent prior to injection (Table III). This approach has been particularly successful in the determination of less volatile pollutants by multiple ion monitoring (MIN); yielding fast analysis time, good precision and efficient separation. Houghton *et al.*[10] have successfully used this method with dodecane as a solvent in the confirmation of synthetic corticosteroids.

TABLE III

SOLVENT SELECTION FOR DIFFERENT INITIAL GC OVEN TEMPERATURES

Final sample solvent	*B.p. (°C)*	*Injection range (°C)*	*GC oven temperature (°C) at injection*
n-Pentane	36	30-50	50
n-hexane	69	65-80	80
Isooctane	99	95-110	100
n-Octane	126	120-135	130
n-Decane	174	170-185	180

However, there is one particular disadvantage in using carriers having high (>100°C) boiling points in direct-interface capillary GC–MS, where the solvent is not diverted at the interface stage, as with the packed-column system. After a small number of injections, traces of solvent remain on the inner surfaces of the analyser and increase the background spectra as the samples are injected. This can be troublesome when decane is used if a full mass spectrum, extending below 120 a.m.u., is required or if the ions monitored, in the MIN mode, are coincident with the solvent spectrum. This was overcome to a large extent by heating the analyser to its maximum working temperature of 100°C. Under these conditions, decane tended to remain in detectable concentrations for 2–3 h. This was not a particular problem for much of the analyses of the environmental samples at this laboratory, as most of the ions monitored have $m/z > 150$. However on such occasions when this did interfere a solvent with lower boiling point, such as octane, was used. This was rapidly pumped away and allowed the normal background subtraction techniques to obtain the spectrum of the chromatographic peaks.

Choice of internal standards

During the past two years, a number of ISs have been incorporated into analytical schemes used at this laboratory[11]. These have now been rationalised to cover the analysis of a large number of persistent pesticide residues and non-volatile industrial pollutants (Table IV). Most of these compounds are readily available, and to date none has been detected in samples taken from Scottish freshwater, coastal and marine environments.

TABLE IV

INTERNAL STANDARDS FOR USE WITH GC–MS, GC–ECD AND HPLC ANALYSIS OF PESTICIDE AND INDUSTRIAL POLLUTANT RESIDUES

Internal standard	*Masses selected (m/z)*	*Elution order on CP Sil 5** (k)	GC–ECD	HPLC–UV	HPLC–fluorescence
[$^2H_8$]Naphthalene	136	3.49	–	+	+
o-Dibromobenzene	236, 238	3.98	+	+	–
2-Bromonaphthalene	206, 208	10.34	+	+	+
4-Bromodiphenyl ether	248, 250	18.72	+	+	–
[$^2H_{10}$]Phenanthracene	188	21.31	–	+	+
2-Bromofluorene	244, 246	26.09	+	+	–
1,2,3,4-Tetrachloro-naphthalene	264, 266	28.43	+	+	+
[$^2H_{10}$]Pyrene	212	32.85	–	+	+
9-Bromoanthracene	255, 258	33.33	+	+	+
[$^2H_{10}$]Chrysene	238	44.07	–	+	+
Methoxychlor	227**	45.99	+	+	–
Mirex	272, 274**	47.56	+	–	–
[$^2H_{12}$]Perylene	264	55.23	–	+	+
Decachlorobiphenyl	495, 498	57.31	+	+	–
Dechlorane	261, 263	–	+	–	–

* Injected at 100°C, 2°C/min.
** Base peak is not the molecular ion.

The ISs are listed in elution order (Table IV) obtained on a 25 m × 0.25 mm I.D. CP Sil 5 fused-silica column and cover the temperature range 80–270°C. The bromohydrocarbons were chosen to cover the lower temperature range, as the lower chlorinated aromatics tend to be more widespread at trace levels in the environment. With the exception of mirex, methoxychlor and dechlorane, they all have an aromatic nucleus and an intense molecular ion. The halohydrocarbons also give a choice of ions in the molecular cluster at M+2, M+4, etc., depending on the number of halogen atoms, which can be particularly useful when it is necessary to avoid a more intense background ion or unresolved contaminant. Of the fifteen ISs selected, ten are suitable for GC–ECD analysis, thirteen are UV absorbing and eight fluoresce.

Originally, aldrin was included as an IS. It is rapidly converted to endrin in the natural environment, and therefore does not persist in its parent form. However, it is stable in the laboratory, both as a solid and in non-polar organic solvents. It was superceded by 1,2,3,4-tetrachloronaphthalene (TCN) which has a very similar capacity factor (k') on the CP Sil 5 column (Fig. 1). TCN has the advantage over aldrin of having intense molecular cluster ions with little fragmentation and of being UV-absorbing and fluorescent.

Methoxychlor and mirex are also two alternatives, the former being preferable. Mirex is used in a number of countries as a pesticide, particularly in the U.S.A. However, it is not used in the U.K. and has never been detected in Scottish samples.

Dechlorane is particularly non-volatile and is useful as an IS when it is difficult to select another standard which would be resolved from other analytes in a crowded chromatogram. Reutergardh[12] has used it successfully in the analysis of toxaphene. It

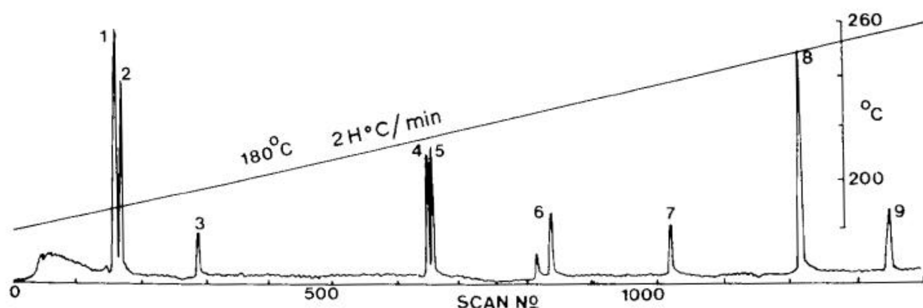

Fig. 1. Chromatogram of a mixture of mothproofing agents and internal standards. Peaks: 1=TCN; 2=aldrin; 3=dieldrin; 4=PAD; 5=mirex; 6=*trans*- and *cis*-permethrin; 7=DCBP; 8=PCSD; 9=dechlorane.

is also valuable in quantifying polybrominated aromatics and aryl phosphates, both of which are eluted between 220 and 270°C.

Multiple internal standards

Most analyses reported to date involving ISs have used a single compound, the response of which is related to all eluents in the chromatograph. This has certainly been sufficiently accurate for most isothermal analyses on packed columns and short analysis times (*ca.* 10 min) in capillary chromatography. However, for multicomponent analyses normally associated with capillary columns one IS does not cover the whole chromatographic range with similar precision. This has been reported by Sauter *et al.*[6] in the analysis of priority pollutants and is further confirmed in our work. A mixture of PAHs and their deuterated analogues were chromatographed with *n*-hexane as solvent, with a 1-μl injection volume at a column temperature of 80°C. The programme rate was 4°C/min after 1 min. There was good agreement between each PAH and its deuterated analogue but a greater disparity as the difference in elution time between the analyte and the selected internal standard increased (Table V).

A similar study was completed for a mixture of mothproofing agents currently being determined in this laboratory. In recent years dieldrin has been replaced by Eulan W.A. New, which has a series of polychlorinated sulphonamido diphenyl ethers

TABLE V

REPRODUCIBILITY FOR POLYAROMATIC HYDROCARBONS AND THEIR DEUTERATED ANALOGUES

Compound	*Weight on column (ng)*	*m/z*	*n*	*Coefficient of variation (2σ)*		
				[$^2H_{10}$] Chrysene RPA	*[$^2H_{10}$] Phenanthracene RPA*	*[$^2H_8$] Naphthalene RPA*
Naphthalene	1	128	10	44		4.4
[$^2H_8$]Naphthalene	1	136	10	43	–	IS
Phenanthracene	1	178	10	8.4	4.9	–
[$^2H_{10}$]Phenanthracene	1	188	10	7.3	IS	–
Chrysene	1	202	10	7.4	–	
[$^2H_{10}$]Chrysene	1	212	10	IS		–

(PCSDs) as active ingredients and a corresponding amine (PAD) as an impurity and as a primary metabolite[4]. Subsequently, a series of *cis- and trans*-permethrin-based mothproofers were also marketed. Since effluents containing these products were discharged to the same catchment, it was desirable to quantify most of these residues in a single chromatogram (Fig. 1). Dieldrin, PADs and permethrin occur in the same clean-up eluate and were analysed together with TCN, mirex and DCBP as ISs.

The results of reproducibility test at two series of concentrations, described as "strong" and "weak" standards, are given in Table VI. The solvent was *n*-decane, 1 μl being injected at a column temperature of 80°C. This was held for 1 min, followed by a programme of 2°C/min. The peak areas and scan times were measured and ratioed against each of the three internal standards, in turn. Both the relative scans (relative retention time) and the peak areas exhibit the same trends. The precision of the measurement declines as the analyte and IS are separated by an increasing time span.

TABLE VI

REPRODUCIBILITY OF PEAK SCAN NO. AND AREA FOR MOTHPROOFING AGENTS WITH DIFFERENT INTERNAL STANDARDS

Compounds in elution order	*Weight on column (ng)*	*m/z*	*n*	*Coefficient of variation (2σ)*					
				TCN		*MIREX*		*DCBP*	
				RSN	*RPA*	*RSN*	*RPA*	*RSN*	*RPA*
Tetrachloro	0.1	266	7	IS	IS	4.1	9.6	4.1	14.8
Naphthalene(TCN)	0.5		7	IS	IS		5.4		11.4
Dieldrin	2.0	277	7	2.9	10.5	0.8	14.0	1.0	13.2
	10.0		7		5.6		7.6		19.5
Mirex	0.5	272	7	4.0	9.4	IS	IS	1.4	12.6
	2.5		7		3.9	IS	IS		13.6
PADs	2.0	321	7	4.2	17.8	0.4	22.0	1.6	13.2
	10		7		6.8		8.9		6.1
Permethrin	0.2	183	7	2.0	28.6	1.0	37.8	0.6	32.4
	1.0		7		10.1		12.3		8.8
Decachloro-	2.0	496	7	4.0	12.5	1.5	12.8	IS	IS
biphenyl(DCBP)	10		7		13.3		15.3	IS	IS

The coefficients of variation of the relative scan number (RSN) for each of the components, when ratioed to TCN as the internal standard, was high (±2.0–±4.0), confirmed that the greatest relative variation in retention time occurred with the peaks which eluted early. This was primarily due to the modification of the column liquid phase during the initial stages of each analysis by the presence of a solvent. The duration of this modification being proportional to the volume of solvent injected[13]. When DCBP was used as the IS, the variation in RSN was considerably reduced, with the exception of TCN (Table VI). The boundary of the solvent modification effects on column retention appeared to be between the elution time for TCN and dieldrin for these chromatographic conditions. If components which are eluted during this initial time span are to be identified by their retention time, it is necessary to incorporate an internal standard in this time span to which they can be normalised.

The variation in the RSNs when ratioed to DCBP and mirex as ISs was much

reduced, except for TCN itself. However, even a variation of ±1.6 % would give a ±14-sec error on a peak that is 18 sec wide at a retention time of 30 min. This would be close to the separation time between two adjacent, resolved peaks and, therefore, care would be needed when identifying compounds, such as individual polychlorinated biphenyl isomers[14]. Although this is not usually a critical factor in GC–MS analysis, as the analytes are not identified on retention alone, it can be important when the spectra are very similar, as with the polychlorinated biphenyls. However, if the retention time of the analytes are close to the internal standard, the accuracy improves to an acceptable level of $<\pm$ 1%. Some of these variations in retention are almost certainly due to the configuration of the Finnigan 9500 oven, which is a floor unit with an internal volume of 56 l. It was originally designed for U-tube packed columns, and is not ideal for capillary column chromatography. However, by using multiple internal standards over a 10-min time span, most of these inherent errors are minimised and good reproducible data are obtained.

In a similar way to the retention time, the coefficient or variation of the relative peak areas (RPAs) increased, in some cases quite markedly, as the time separation between analyte and IS was increased. This held true for each series of data normalised to TCN, mirex and DCBP in turn, and it was shown more clearly with the higher concentrations of the "strong" standard, injected on-column (Table VI). When a retention time span is imposed so that dieldrin is quantified relative to TCN (coefficient of variation. C.V. = ±5.6%), PAD is quantified relative to mirex (C.V. = ±8.9%) or DCBP (C.V. = ±6.1%) and permethrin quantified relative to DCBP (C.V. = ±8.8%) then good reproducible data are obtained over a 30–40 min chromatogram. At the lower concentrations, $<$ 1 ng on-column, the reproducibility declines, particularly for permethrin. This is simply due to losses by inclomplete transfer and adsorption during injection and chromatography, and due to the ion statistics of the trace components in the mass spectrometer. These concentrations are at the lower limit at which any quantitation may be seriously contemplated, although they are above the qualitative detection limit of 3 × baseline noise. The "strong" and "weak" standards bracket the range of concentrations that would be expected in the actual samples received. Increased precision, or lower detectable limits at the same accuracy, may be obtained with the on-column injector by increasing the injection volume to its maximum value of 5μl for this system, and/or by decreasing the final volume of the sample.

CONCLUSIONS

(1) Following the retrofit of the on-column injector to the GC–MS instrument, superior quantitative results were obtained compared with the splitless injector, particularly for less volatile, non-polar herbicides and pesticides.

(2) The final sample solvent can be selected on the basis of the desired chromatographic separation to give shorter analysis times and minimal delay between injections.

(3) The precision in peak retention times and areas is markedly improved by the use of multiple internal standards, eluted within 10 min of the analyte.

(4) Many of the internal standards selected may be used with GC–MS, GC–ECD and HPLC with UV and fluorescence detection.

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GLOSSARY

De-ethyl terbutryn	4-Amino-2-methylthio-6-*tert.*-butylamino-1,3,5-triazine
Dieldrin	Hexachloroepoxyoctahydrodimethanonaphthalene
Methoxychlor	2,2-Bis(*p*-methoxyphenyl)-1,1,1-trichloroethane
Mirex	Dodecachloropentacyclodecane
PAD	Polychloro-2-aminodiphenyl ether (major constituent, pentachloro isomer)
PCSD	Polychloro-2-chloromethylsulphonamidodiphenyl ether (major constituent pentachloro isomer)
Permethrin	3-Phenoxybenzyl-*cis*- *or* *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
Terbutryn	4-Ethylamino-2-methylthio-6-*tert.*-butylamino-1,3,5-triazine
Toxaphene	A mixture of polychlorinated camphenes

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