During our visits to the field laboratories and in our communications with them, we frequently hear, "We don't have time to spend on all of this quality assurance work. It cuts sharply into our production". The answer is, of course, "we do not have time *not* to perform the quality assurance checks". Of what value is it to turn out reams and reams of data if we do not have the assurance, other than a gut feeling—assurance with facts and figures that the data obtained are accurate and reliable?

In addition to the above, another safeguard FDA applies to its use of analytical methods is the requirement that if a product is found by analysis to be in violation of the FD&C Act, a second or "check" analysis has to be performed. The check analysis has to be run by a second analyst. The check analyst starts from scratch making his own composite, if one is required, checking his own standards, solutions, etc. Only if the results from the two analyses are in reasonable agreement is regulatory action considered against the product. Any method used which is not official must be validated in the hands of the analyst by recovery studies or other appropriate studies before the results by the method are acceptable. We take into account the fact that frequently such a quantitative method is not absolutely definitive, and we must follow the quantitative analysis with a confirmative step such as mass spectrometry or thin-layer chromatography. We must be as certain as possible that the ingredient we are quantitating is in fact the ingredient we think it is.

So goes the battle in FDA's regulatory laboratories.

New Approaches to FDA Analytical Problems†

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The formulation of a molecular weight listing of pesticides and industrial chemicals is described. Application to residue analysis by mass spectrometry is reported. A novel approach to quantitation of polychlorinated biphenyls is presented.

INTRODUCTION

During normal surveillance analysis for pesticide residues, compounds are frequently encountered which cannot be readily identified by available gas chromatographic data. In the past, identification was attempted using different detectors and stationary phases in the gas chromatographic separation in the hope that the combined retention data acquired might correspond to an already characterized pesticide or industrial chemical. Such cross correlations of retention data are time-consuming and rely heavily on the recreation of the standard conditions under which the original data for comparison was formulated. Furthermore, the compilation of a substantial retention data base for this type of approach to identification is a long-range undertaking to achieve absolute completion.

BACKGROUND

More recently, with the acquisition of combined gas chromatography–mass spectrometry (GCMS) systems in seven FDA field laboratories, the structural elucidation of detected unidentified compounds by conventional GC has been referred directly to GCMS techniques. The essence of this approach to structural elucidation, especially with low-resolution instruments, is knowledge of the molecular weight of the compound under investigation. In particular, chemical ionization (CI) techniques often provide conclusive evidence for the recognition of the so-called quasi-molecular ion or protonated molecular ion. This single piece of information alone can narrow down the possibilities provided a comprehensive listing of pesticides and industrial chemicals is available, organized according to molecular weight. No commercially available compilation[1] exists which contains within its database all the compounds pertinent to FDA analyses.

MOLECULAR WEIGHT LISTING OF PESTICIDES AND INDUSTRIAL CHEMICALS

The need for a listing containing all the possible compounds that might be encountered in regulatory analyses is long overdue. To meet this demand, such a database has been initiated and at the moment the listing containing 1650 compounds has been computer sorted according to molecular weight (Figure 1). The identification code refers to the

MOLECULAR WEIGHT LISTING OF PESTICIDES AND INDUSTRIAL CHEMICALS

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MW	COMPOUND NAME	FORMULA	IDENT
53 0265	ACRYLONITRILE	C3 H3 N	AEN
56 0262	ACROLEIN	C3 H4 O	ACY
58 0419	ALLYL ALCOHOL	C3 H6 O	ALP
62 0368	ETHYLENE GLYCOL	C2 H6 O2	
64 0037	SODIUM CYANAMIDE	C H N2 NA	0025
73 0891	SEC-BUTYLAMINE	C4 H11 N	0482
76 0627	B-HYDROXYETHYLHYDRAZINE	C2 H8 N2 O	027BHYZ
77 0277	FLUOROACETAMIDE	C2 H4 F N O	FLR
84 0436	AMITROLE	C2 H4 N4	0084AMT
86 0368	2-BUTYNE-1,4-DIOL	C4 H6 O2	BYY
88 0524	ETHYL ACETATE	C4 H8 O2	ETF
92 0029	1-CHLORO-2,3-EPOXYPROPANE	C3 H5 CL O	EPI
94 9622	CHLOROACETIC ACID	C2 H3 CL O2	CAA
94 0521	AVITROL 100	C5 H6 N2	0947AMN
97 9690	1,2-DICHLOROETHANE	C2 H4 CL2	
99 0141	ALLYL ISOTHIOCYANATE	C4 H5 N S	ALT
99 9906	SODIUM MONOFLUOROACETATE	C2 H2 F O2 NA	0267SMF
102 0252	ETHYLENE THIOUREA	C3 H6 N2 S	0295NAU
105 4822	CIS-3-CHLOROACRYLIC ACID	C3 H2 CL O2	CPY
106 0419	BENZALDEHYDE	C7 H6 O	BEY
107 9978	3-CHLOROPROPIONIC ACID	C3 H5 CL O2	CPF
109 9690	1,3-DICHLOROPROPENE (D-D)	C3 H4 CL2	0059DIP
110 0268	CATECHOL; O-DIHYDROXYBENZENE	C6 H6 O2	CCL
111 0085	AMINOMETHYLPHOSPHONIC ACID	C H6 N O3 P	0585
111 9684	N-VINYL-2-PYRROLIDONE	C6 H9 N O	1200
111 9847	1,2-DICHLOROPROPANE (D-D)	C3 H6 CL2	0059PDC
112 0000	CHLOROBENZENE	C6 H5 CL	0245
112 0273	MALEIC HYDRAZIDE	C4 H4 N2 O2	0084MHY
114 0099	AMMONIUM SULFAMATE	H6 N2 O3 S	0005
120 0067	KOPAR	C3 H6 CL N O2	0181KOP
123 9847	DICHLOROBUTENES	C4 H6 CL2	DHE
128 0626	NAPHTHALENE	C10 H8	
148 9681	VAPAM; METHAM-SODIUM	C2 H4 N S2 NA	0156MES
128 9981	4-CHLOROPYRIDINE-N-OXIDE	C5 H4 CL N O	CPI
130 1358	ISOOCTYL ALCOHOLS	C8 H18 O	1031
131 9300	1,1,1-TRICHLOROETHANE	C2 H3 CL3	0193TRI
133 0640	2-AMINOBENZIMIDAZOLE; METAB. OF BENOMYL	C7 H7 N3	0411BEI
135 9524	EPIBROMOHYDRIN	C3 H5 BR O	1205
137 9662	CHLORYLIC ACID	C2 H7 O2 AS	0400CAC
139 9455	METHYL ARSONIC ACID	C H5 O3 AS	0420MAA
141 0012	MONITOR	C2 H8 N O2 P S	0480MEH
141 9185	1-BROMO-2-CHLORO-ETHANE	C2 H4 BR CL	ETX
141 9588	DALAPON	C3 H4 CL2 O2	0030HL
141 9952	CHLOREX; BIS-(2-CHLOROETHYL)ETHER	C4 H8 CL2 O	0180CHI
142 0185	4-CHLORO-O-CRESOL; METABOLITE OF MCPA	C7 H7 CL O	0562
142 0185	4-CHLORO-M-CRESOL	C7 H7 CL O	CIE
142 9541	ETHIDE; 1,1-DICHLORO-1-NITROETHANE	C2 H3 CL2 N O2	0052ETD
143 9742	ETHEPHON	C2 H6 CL O3 P	0425ETH
145 0021	PRYNACHLOR HYDROLYSIS PRODUCT	C10 H11 N	0489
145 9690	M-DICHLOROBENZENE	C6 H4 CL2	0244
145 9690	O-DICHLOROBENZENE	C6 H4 CL2	0190DHB
145 9690	PARACIDE; P-DICHLOROBENZENE	C6 H4 CL2	0191PRD
146 0044	ACROLEIN PHENYLHYDRAZONE	C9 H10 N2	ACZ
146 1307	2-ETHYL-1,3-HEXANEDIOL	C8 H18 O2	EUX
147 0810	CARVONE; P-METHA-6,8-DIEN-2-ONE	C10 H14 O	CCF
148 0888	ANETHOLE; 1-METHOXY-4-PROPENYLBENZENE	C10 H12 O	ANE
149 0607	CDEA	C6 H12 CL N O	0189CDE

Figure 1. A typical page from the Molecular Weight Listing of Pesticides and Industrial Chemicals.

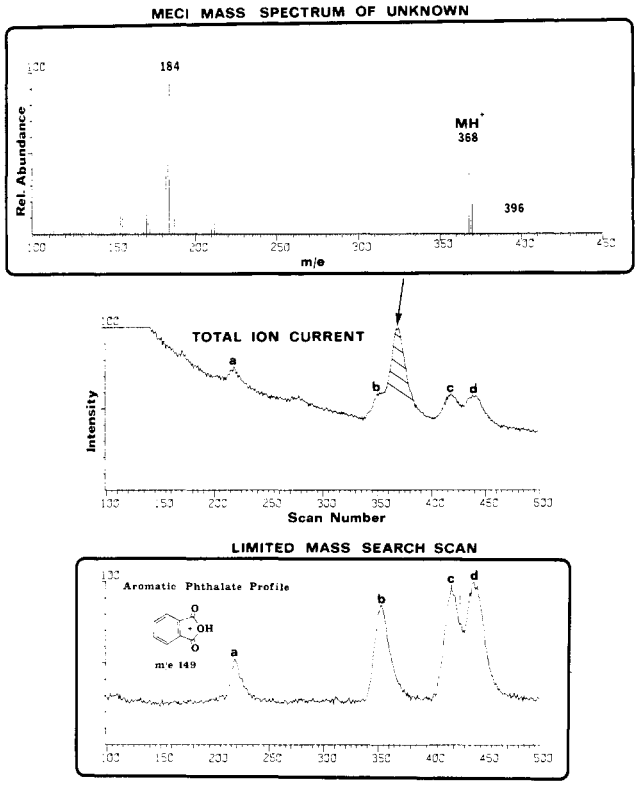

Figure 2. GCMS residue analysis of cherries.

"Primary Standard Data Sheet" provided with each standard supplied to FDA personnel. The three letter code appearing after the numerical code is from the "Nanogen Index".[2] Both these codes are references which provide additional details on structure, toxicity, usage, etc. Although the major anticipated use of this list involves mass weight to the nearest integral whole number, exact molecular weights to the fourth decimal place have been deliberately incorporated to aid in high resolution measurements and mass defect determinations. As each of these compounds or their metabolites are recorded by an FDA mass spectrometrist, the resulting mass spectral data (EI, CI, FI, or FD) will be numerically included (ten strongest peaks and intensities) in the listing and concurrently printed up as a plotted normalized spectrum for a master compilation of reliable mass spectral data. It is planned to develop further indices to this master compilation by name and molecular formula. The unique feature of this database of mass spectral information which differentiates it from any other is that the pesticides and industrial chemicals to be encountered have been previously identified and sorted by molecular weight without waiting for the characteristic mass spectrum.

EXAMPLES

Case 1. Residue Analysis of Cherries. To illustrate the potential of such a generated database, a recent example of an unidentified peak or eluting compound from the residue analysis of fresh Bing cherries was quickly solved by reference to the molecular weight listing of pesticides and industrial chemicals. The total ion chromatogram (TIC) (Figure 2) indicated a number of peaks in the sample. However, by the simple computer technique of searching each mass scan for only m/e 149, an ion highly characteristic of phthalates, it was clear that peaks a, b, c, and d belonged to this class of compound. The resulting mass spectrum of the unknown revealed that it had a molecular weight of 367. Positive identification of the molecular weight was observed by the presence of an adjunct ion at m/e 396 corresponding to $(M + 29)^+$ resulting from an ion–molecule collision with $C_2H_5^+$ formed by high-pressure methane collisions. In addition, the presence of an ion at m/e 370 with approximately 30% of the intensity of the MH^+ ion at m/e 368 was strong indication that the molecule possessed one chlorine atom. A quick visual search of the molecular weight listing indicated that the

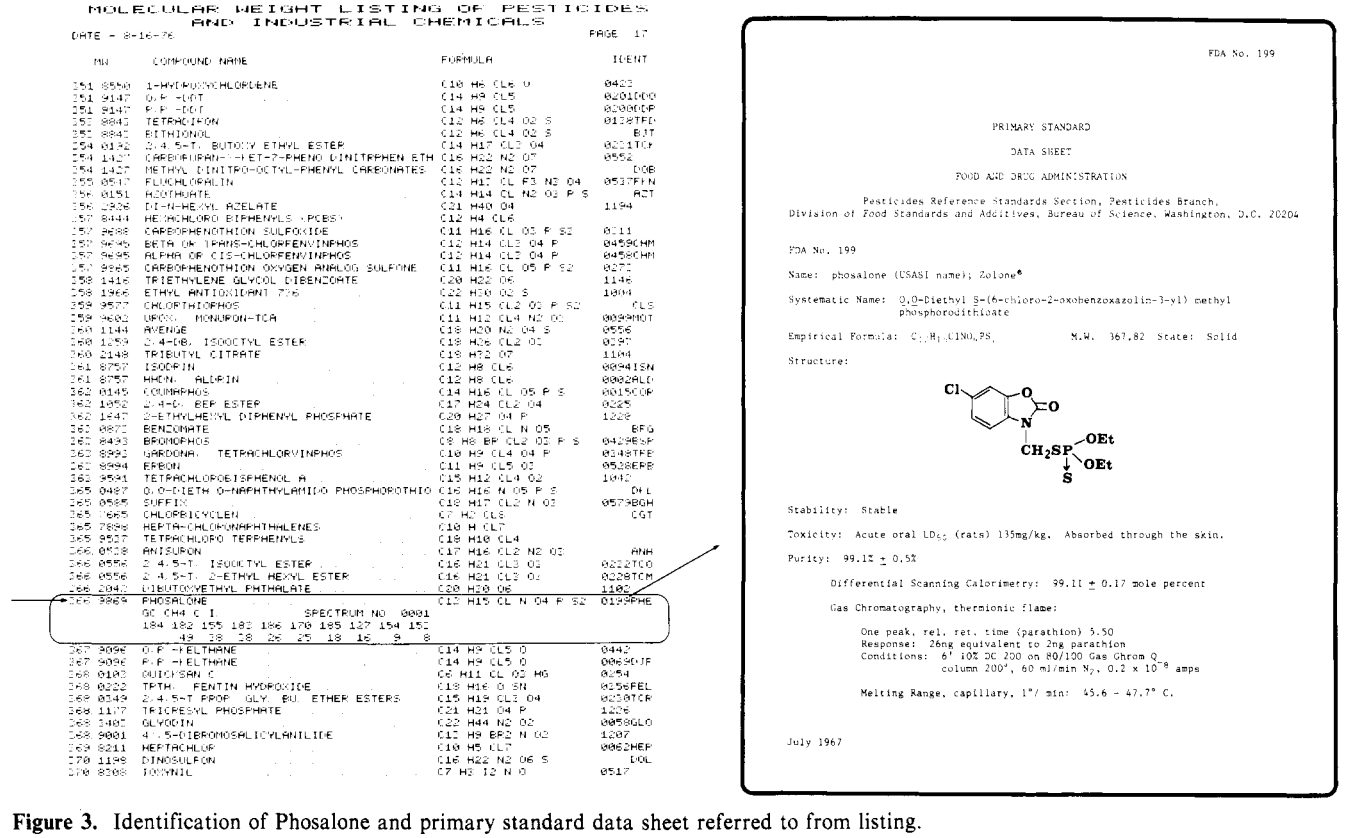

Figure 3. Identification of Phosalone and primary standard data sheet referred to from listing.

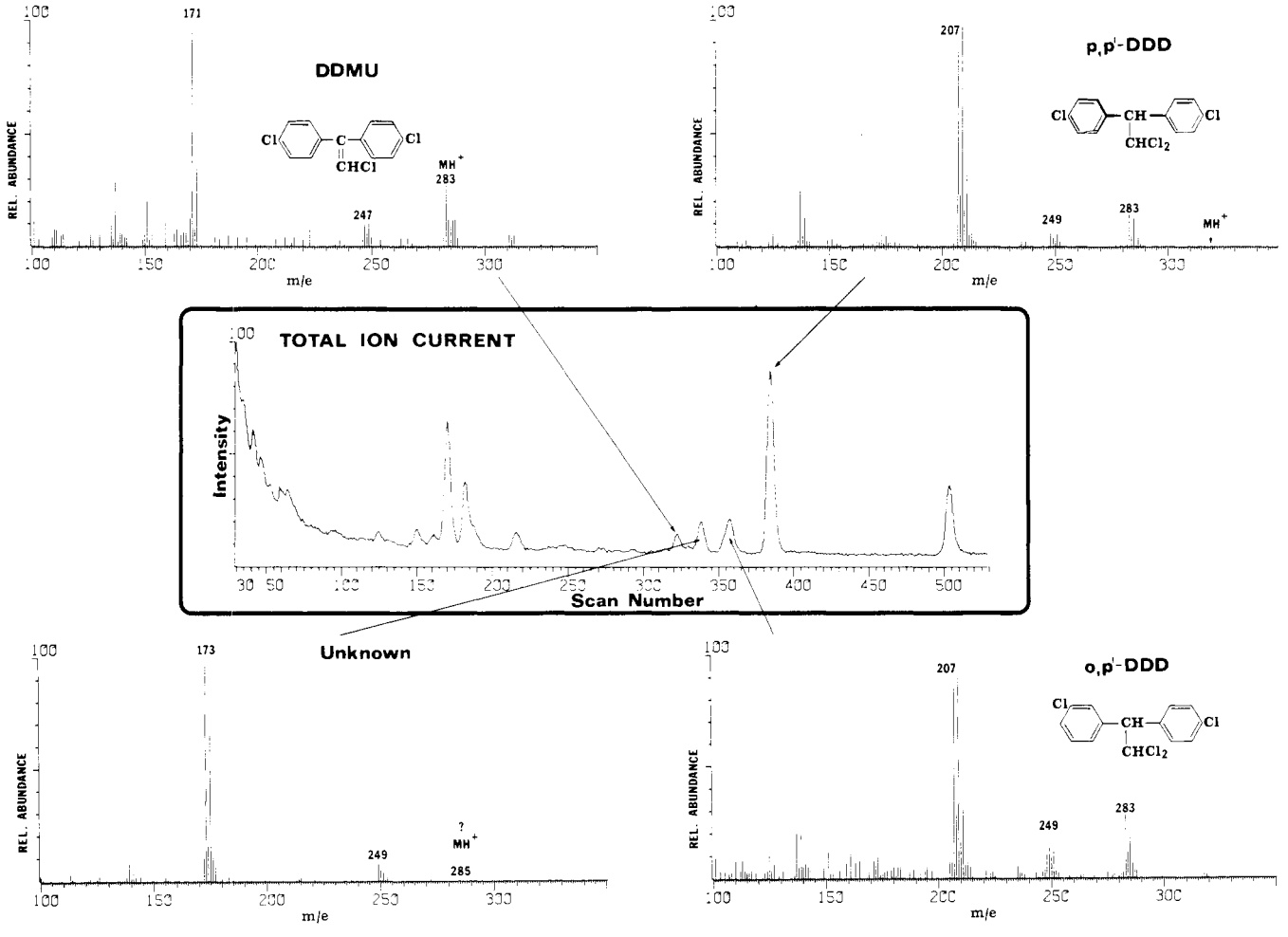

Figure 4. GCMS residue analysis of fresh carp.

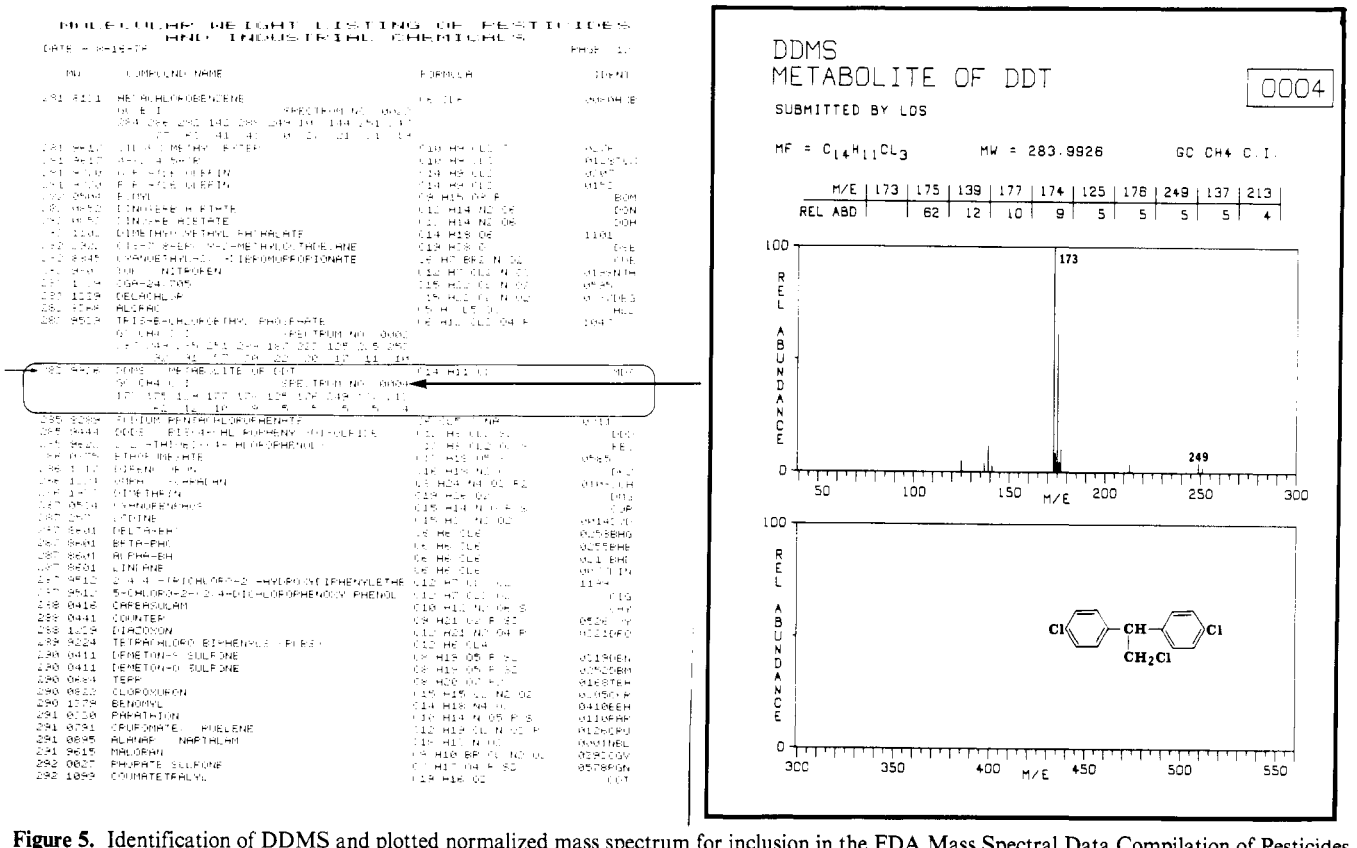

Figure 5. Identification of DDMS and plotted normalized mass spectrum for inclusion in the FDA Mass Spectral Data Compilation of Pesticides and Industrial Chemicals.

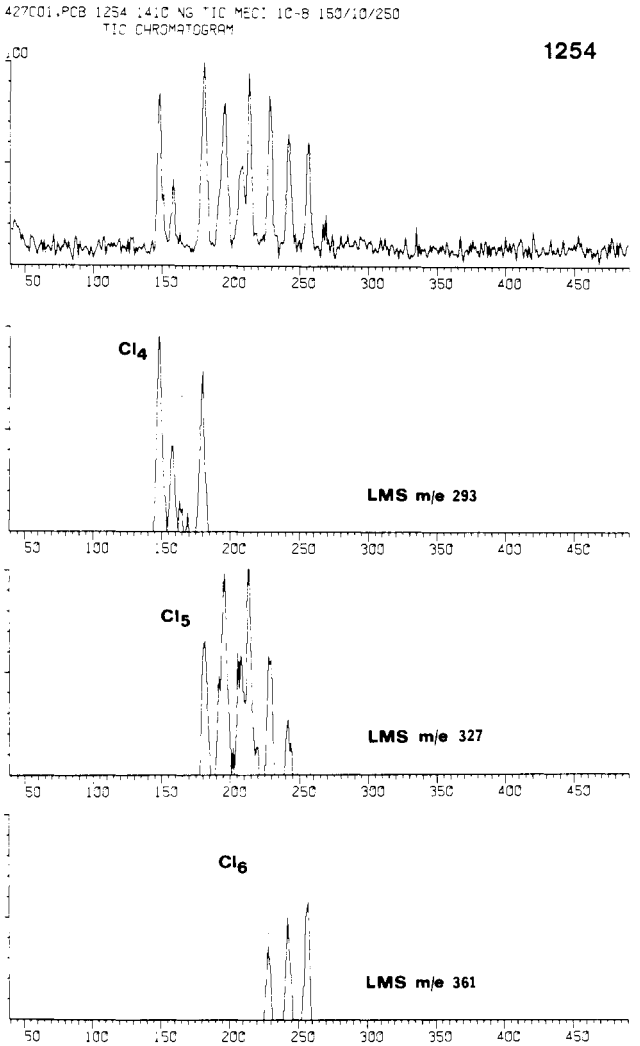

Figure 6. GCMS analysis of Aroclor 1254 and subsequent limited mass scan searches for chemical profiling.

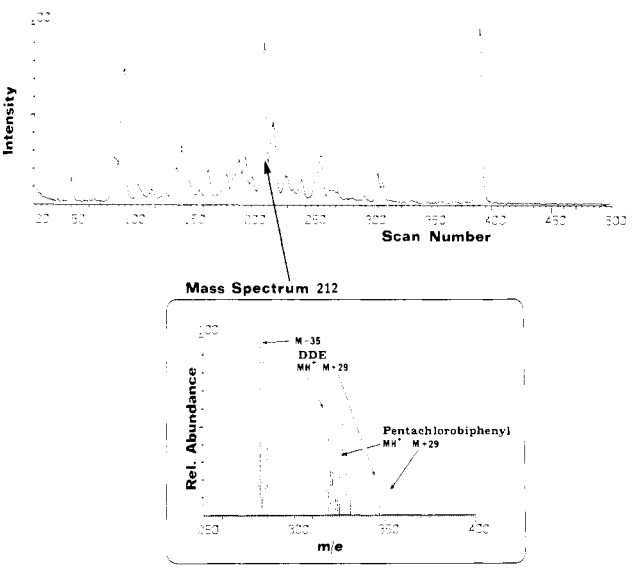

Figure 7. GCMS analysis of salmon extract illustrating co-eluting compounds at scan 212.

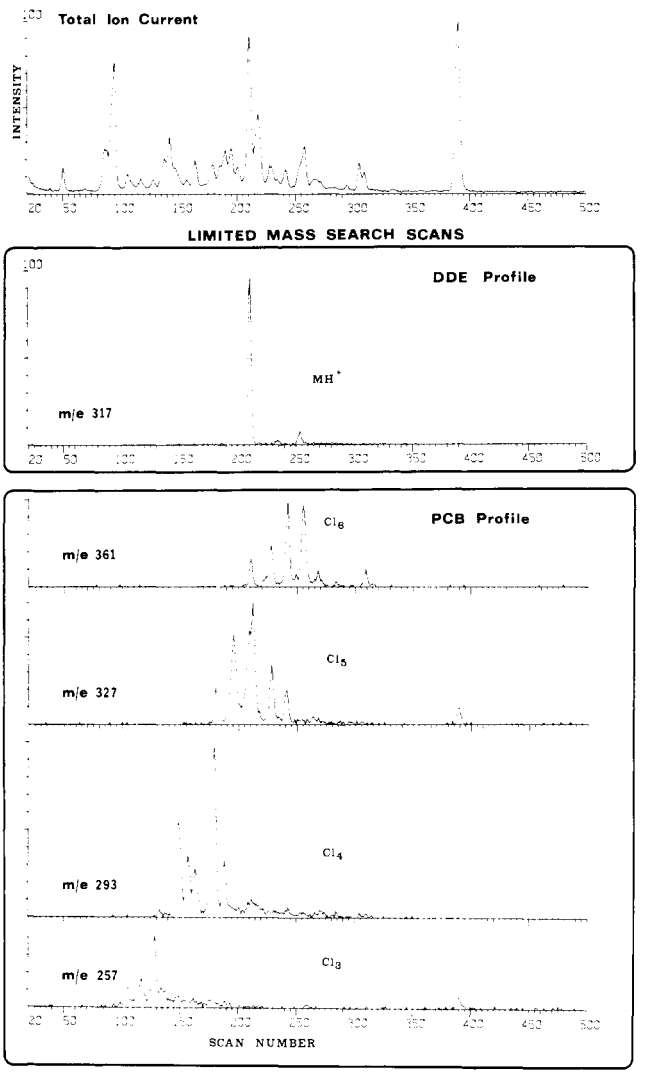

Figure 8. GCMS analysis of salmon extract and limited mass scan searches to profile PCB's and DDE.

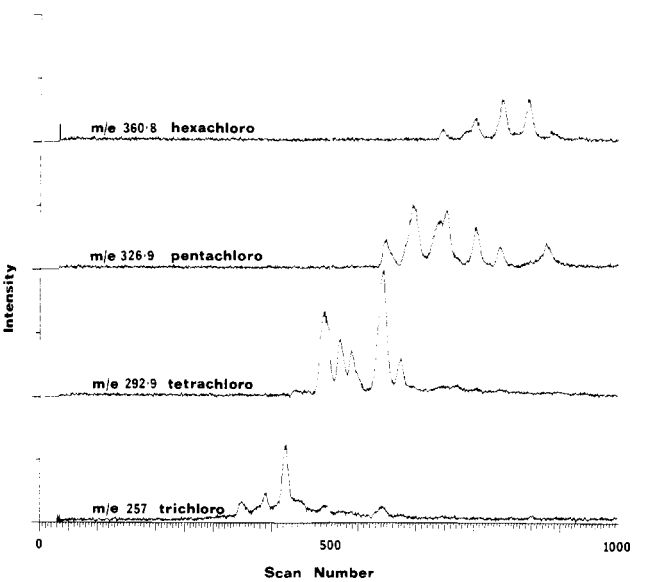

Figure 9. GCMS-CI-SIM analysis of salmon extract.

compound was Phosalone (Figure 3). Unambiguous identification was provided by recording a standard reference sample under exactly similar conditions as the sample. Both the retention time and mass spectrum agreed. The unknown had been quickly solved. A point worthy of mention is that a solution was arrived at by a low-resolution instrument. Normally, such a complex molecule containing N, O, S, P,

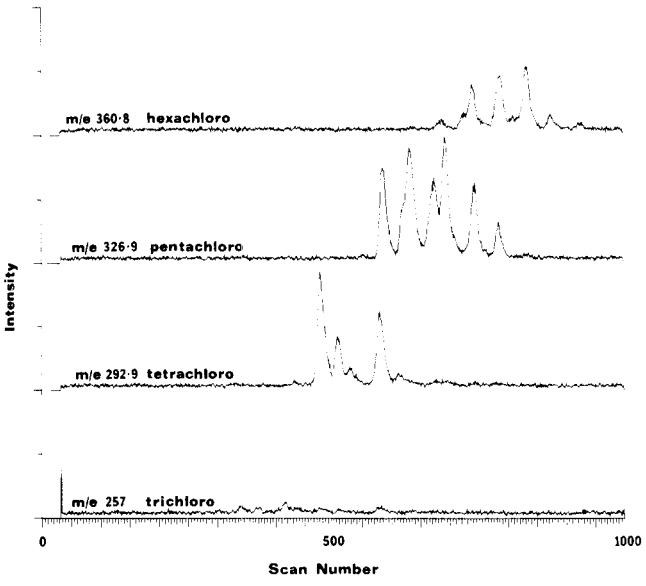

Figure 10. GCMS-CI-SIM analysis of Aroclor 1254.

and Cl would have required high-resolution measurements to suggest plausible structures.

Case 2. Residue Analysis of Fresh Carp. Sometimes the mass spectrum of a compound under investigation has not yet been added to the database although the molecular weight, molecular formula, etc., have already been added. Such information can provide clues to the possible identity of the unknown. To illustrate this additional facet of the uncompleted database, an unidentified compound was encountered in the analysis of fresh carp. A number of peaks had already been identified by existing GC using electron capture detector (EC) systems. In this particular case the unknown was strongly suspected of being a metabolite both by its retention time and close similarity in mass spectral characteristics to the identified metabolites of DDT found present, DDMU and DDD (Figure 4). By inference, the molecular weight was calculated to be 284 since the prevailing fragmentation of this type of structural entity was to lose a chlorophenyl group, i.e., loss of 111 amu. A search of the listing (Figure 5) revealed that the possible metabolite could be DDMS. Inspection of an available standard of DDMS proved the case and the spectrum was then added to the list and compilation for future reference.

In summary, the construction of a master listing of pesticides and industrial chemicals used today together with their respective mass spectra can provide a powerful tool to aid in the structural elucidation of unknowns found in regulatory samples. If the compound is not on the list then at least a number of possibilities have been instantly removed from consideration and further detailed study will be necessary to solve the identity of the compound. This screening process provides either quick solutions or difficult problems requiring extended study enabling the analyst to make decisions on time and effort to be expended.

CHEMICAL PROFILING OF POLYCHLORINATED BIPHENYLS

Quantitative analysis of polychlorinated biphenyls (PCB's) has been a difficult problem since their detection in the environment by Jensen[3] in 1966, primarily because of the large number of compounds involved. A novel approach recently experimented with PCB residue samples has been the chemical profiling of the PCB content in the sample to ascertain the most suitable available standard to be used for subsequent quantitation even in the presence of interfering substances. The essence of the success of this approach was based on the observed fact that PCB's on CI produce almost entirely the molecular ion cluster. Since only molecular ions are formed, limited mass scans (LMS) can be conducted using the data system to profile the PCB content by specifying those ions corresponding to monochloro, dichloro, trichloro, etc. The resulting LMS searches were found to be highly characteristic in differentiating the various Aroclor standards available (Figure 6) and could be effectively used as chemical profiles to chose which Aroclor standard was most suitable for quantitation of a residue sample. To illustrate the real world problems encountered in the analysis of PCB's in fish samples (Figure 7), the mass spectrum represented by scan 212 in the TIC chromatogram indicated that this particular peak contained two co-eluting compounds—DDE and a pentachlorobiphenyl. Such interferences make selection of a suitable standard for quantitation difficult and impose further chemical separation and clean-up procedures. However, by computer techniques on this TIC chromatogram, LMS searches were able to pull out selectively both the PCB and DDE profiles (Figure 8). The observed profile for the salmon extract was similar to that previously observed for Aroclor 1254. Certain discrepancies existed in the profiles which could only have resulted from weathering and/or metabolism.[4] However, the overall profile closely resembled the profile observed for Aroclor 1254. To achieve quantitation without further chemical treatment of the sample the extract was rerun using single ion monitoring (SIM) techniques (Figure 9) and the areas under each ion value were calculated and compared with those observed for a standard Aroclor 1254 sample (Figure 10). The calculated value of 14 ppm agreed with the value using conventional GC with EC.

CONCLUSIONS

With the experience and positive reactions gained from the molecular weight listing project it is anticipated that additional compounds and metabolites will be added to the list as they are reported in the literature. A deliberate attempt to record all the mass spectral data is currently underway as a joint effort within FDA laboratories. At the same time, available mass spectral data are being forwarded to EPA for inclusion in the Cyphernetics MSSS file system.[1]

In the case of PCB quantitation, plans are already underway to refine the chemical profiling techniques and quantitate on the basis of individual isomeric groups (mono, di, tri, etc.) rather than related to an Aroclor standard mixture.

ACKNOWLEDGMENT

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LITERATURE CITED

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(1) R. S. Heller, G. W. A. Milne, R. J. Feldmann, and S. R. Heller, "An International Mass Spectral Search System (MSSS). V. A Status Report", *J. Chem. Inf. Comput. Sci.*, **16**, 176 (1976).
(2) K. Packer, "Nanogen Index. A Dictionary of Pesticides and Chemical Pollutants", Nanogens International, Freedom, Calif. 95019, 1975.
(3) S. Jensen, "Report of a New Chemical Hazard", *New Sci.* **32**, 612 (1966).
(4) L. D. Sawyer, "Collaborative Study of the Recovery and Gas Chromatographic Quantitation of Polychlorinated Biphenyls in Chicken Fat and PCB-DDT Combination in Fish", *J. Assoc. Off. Anal. Chem.*, **56**, 1015 (1973).