microbiological methods. The final recommendations of the subcommittees are voted upon by the Association as a whole. This voting, however, is restricted to those organizations, federal or state, who exercise official control over the commodity under consideration.

Although this mechanism may appear rather cumbersome, it usually works very smoothly, since disagreements are worked out at the preliminary stages before it becomes necessary to take a formal vote. The restriction of voting privileges to government officials has not proved objectionable in practice to either industry or consumers, since reports of projected and completed projects are delivered at the annual meetings and published in the *Journal of the Association of Official Analytical Chemists* to provide an opportunity for comment and second guessing by all who are interested.

The insistence of the AOAC on a demonstration of the practicality and reliability of the methods it approves has clearly placed its methods in a position to obtain legal endorsement. At a meeting of an ad hoc FAO/WHO Committee of Experts on the selection of Referee Methods for the determination of contaminants in foods,[4] AOAC methods were chosen for endorsement almost exclusively, since they were the only methods in the world providing a published basis for judging suitability that were available to the scientific and legal professions. As an outgrowth of the experts' meeting, the methods that were selected can be used to develop a set of criteria which may serve as the basis for selection of referee methods at trace levels which are suitable for use in preventing or settling disputes in international trade: These are:

Accuracy: at least 70% recovery at the 10 ppb level

Precision: less than 40% coefficient of variation between laboratories

False positives and false negatives: not more than one incorrect decision per unit of 10 decisions (samples and/or laboratories)

Further discussions are required to develop criteria for: (1) ratio of a measurement to that of the *blank* (as either a signal-to-noise ratio or an absolute magnitude of the blank relative to the measurement); (2) *sensitivity* of measurements (discrimination between measurements or measurement per unit concentration); and (3) *limit of detection*—lower limit of reliable measurement.

The FDA also utilizes the methods of other organizations, usually in more specialized areas. With polymers, the extensive American Society for Testing and Materials specifications are useful in characterizing physical and chemical properties. The methods of the American Oil Chemists' Society and the American Association of Cereal Chemists also provide useful methods in their areas of expertise. These organizations attempt to maintain uniformity in method of analysis where common methods are required for their individual memberships.

To summarize this final section, FDA utilizes the collaborative study mechanism of the AOAC to provide validated methods of analysis needed by governments for the enforcement of the law and by industry for compliance with the law.

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Application of Tolerances, Standards, and Methodology in the Enforcement of the Food, Drug, and Cosmetic Act†

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The FDA quality assurance program for its 20 field laboratories, the use of standards established by officially recognized organizations, the national check sample program, and the quality assurance audit visit program are described.

At first glance it would appear that there is not very much that needs to be said under this topic. The Food and Drug Administration as a regulatory agency covering the interstate commerce of foods, drugs, and cosmetics, as well as medical devices and diagnostic products, is a scientific information-consuming agency. As such, it utilizes the analytical methods and standards developed both by itself and the outside scientific community for obtaining data to determine whether any regulatory action is indicated. Nearly every action which FDA takes is based on data provided by its laboratory personnel—in large measure by its analytical chemists. FDA employs some 900 chemists, at least half of whom are analytical chemists.

Having said the above, what remains to be said? Actually a great deal, not in terms of volume but in terms of importance. A former commissioner of FDA has said, "The public interest simply cannot be served if our regulatory decisions are not supported by sound science". Properly validated, sensitive analytical methodology and published standards are essential for the proper administration of the Food, Drug and Cosmetic Act. The question always has to be asked—how reliable are the data coming in from various laboratories? How comparable are the data from one laboratory with those from another? The mercury–seafood contamination problem was an outstanding example. The discovery of mercury in Great Lakes fish was made by the University of Western Ontario utilizing neutron activation analysis. By this time it was known that it was not elemental mercury that posed the problem in fish, but rather the monomethylmercuric ion formed by biological conversion. By this time, it was also obvious that older analytical procedures such as the dithizone colorimetric method were not adequate, and a flurry of analytical reports utilizing

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a variety of methods were received from all over the world. Could all of the data be utilized? How valid were the data and analytical methods used to obtain the data? A regulatory agency such as FDA had to have a recognized, "official" method which all concerned laboratories could use and all data so obtained be comparable. Such a method was finally obtained by utilizing the collaborative process through the AOAC described to you by Dr. Horwitz in the preceding paper. This process resulted in official adoption by the AOAC of the cold vapor atomic absorption method for mercury in fish. The official method now provided the common ground needed to compare results from laboratories around the world. This method was and is the standard against which we compare mercury results obtained by other methods in non-FDA laboratories. Without this standardized method, we would not have been able to cope with the tuna–swordfish problem which represented a near-crisis drain on FDA resources in early 1971.

FDA has the same need for standardized analytical methodologies and measurement procedures for the monitoring of thousands of market place products. FDA regulates a large segment of American industry—200 to 300 billion dollars worth. The products are of ever-increasing diversity and the analytical problems they present cover the spectrum. The samples to be analyzed may be relatively pure compounds, as is the case of many drug preparations, or very complex mixtures, as is the case with foods. The chemical component to be measured may constitute the bulk of the sample or it may merely be a parts per billion trace. Further, the large number of measurements necessary for routine monitoring requires that sample preparation and analysis be operationally simple processes amenable, wherever possible, to computer-assisted analysis and automation.

Of course, a regulatory agency such as the FDA must take into account the fact that the process of measurement is statistical in nature. It is in the nature of things that a very small portion of uncertainty attaches to the result of every process of measurement, regardless of the degree of excellence of analytical practice and the quality of the employed measuring devices. Uncertainty is a fundamental attribute of natural phenomena and must be contended with in every process of measurement. This fact of life is taken into account when standards and tolerances are set. For instance, the ranges of not less than such and such a percentage and not more than such a percentage of declaration given in the USP and NF monographs are set up to take into account the errors inherent in the analytical method, the expected variability between analysts, and the expected variability between laboratories. The tolerance for the pesticide Malathion on apples is 8 ppm. This does not mean, of course, that apples become unsafe at 8.1 ppm Malathion. Such a limit is specified as a result of toxicological and analytical data, allowing for errors in measurement, submitted to the regulatory agency which show that the established tolerance is a reasonable dividing line for the intended purpose. The USP and the NF and the analytical procedures they contain are recognized as official in the Food, Drug and Cosmetic Act itself. The methods recognized by the AOAC have been declared to be official for purposes of enforcement of the FD&C Act by regulations promulgated by FDA.

How does FDA attempt to assure that the analytical methods available to it are carried out properly in our laboratories? Inaccurate measurements may result in serious health, legal, or economic consequences. We know that our magic instrument boxes can lie to us if not kept honest by proper calibration, standardization, and tender loving care. We know that the analytical method is made up essentially of two halves: one consists of the measurement procedures, i.e., the means of analysis of the composition and the properties of matter; the second is the human half—the contribution of the analyst. To properly keep each of these two halves at an accurate, precise, and high-performance level, a laboratory quality assurance program must continually be carried out. Just as eternal vigilance is the price of liberty so a continuing laboratory quality assurance program is the eternal price of a regulatory agency's credible laboratory data collection.

FDA has had a formal QA program in effect since 1966. The FDA quality assurance program for its 20 field laboratories consists essentially of three parts:

1. An internal individual laboratory quality assurance program modeled after a program suggested by Headquarters. The local program is put into writing, a copy of which is provided to Headquarters.
2. A national check sample program consisting of samples sent as "unknowns" to the field laboratories.
3. A QA audit visit to each field laboratory approximately once a year.

The objectives of the program are:

1. To upgrade the overall quality of laboratory performance.
2. To help ensure that our analytical work will withstand legal scrutiny in regulatory actions.
3. To detect training needs.
4. To measure the precision and/or accuracy of analytical results among analysts within a laboratory and between laboratories.
5. To provide a permanent record of instrument performance.

The internal QA portion of the overall QA program sets up the criteria for checking analytical performance as well as equipment, instrumentation, standards, and the time frames for accomplishing the checking.

The internal quality assurance program for the equipment and instrumentation calls for:

1. Performance criteria for each instrument.
2. Performance checks (including what the check is and how it relates to performance criteria). The required frequency for each check.
3. Maintenance requirements (both routine in-house and by a qualified repairman). The required frequency for each maintenance check.
4. Record of malfunctions and repairs.
5. Specific action to be taken where instrument fails to meet performance criteria.

We also have to consider standards. For instance, how does the analyst know that what is written on the bottle label is actually that which is in the bottle? Normally, NF, USP, Bureau of Standards, ASTM, and pesticide standards from EPA are accepted as received since these have been previously tested by an officially recognized organization. It is left to the analysis itself to detect a problem in those rare instances where there may be one. With all other standards, the first analyst to open the bottle must run some kind of appropriate validating test or combination of tests, i.e., IR or UV curve, melting point, composition analysis, or what have you, to assure himself that the label declaration properly reflects the identity and composition of the material. These data are recorded in a notebook with the date and name of the analyst. Whenever the same standard is used at a later date, a run of the same test(s) as when originally checked will quickly show if there has been any deterioration or other change. No test or assay based on a comparison to a secondary standard (working standard) can be considered acceptable unless the secondary standard has been evaluated and a written record exists of the evaluation.

The internal quality assurance program calls for a review of analytical worksheets to be periodically undertaken by laboratory supervisors with the number of worksheets and

1. Is the method used an official method for this product (i.e., U.S.P., N.F., AOAC)? ____
2. If so, was there any deviation from the official method? ____
3. If the method used on this product is not an official method for this product, did analyst validate his method on the product by either recovery studies or by use of a complete ____
4. If there was any deviation from an official method for an official product is an acceptable reason given for deviating from the official method? ____
5. Has any standard other than an official reference standard been used in any of the assays involved here? ____
6. If so, how was its suitability for use -- i.e., identity, potency, and purity -- established? ____
7. Are the attached worksheets the original record of all work done on and in connection with the analysis of this sample?

Figure 1. QA review of analytical worksheets.

frequency of review being specified. The review is aided by a check list checking for such items (see Figure 1). In contrast to research work, data from the analysis of a regulatory sample are not put into a bound notebook but must be placed directly on the analytical worksheet. The data cannot be put on a piece of paper and then transcribed to the worksheet. The worksheet must be the *original* record.

As mentioned previously, one very critical item to be checked by a regulatory laboratory is the use or non-use of an official method. Analytical worksheets indicating that "essentially" the official method was used or that a "modified" method was used must be regarded as a warning signal. Such statements require an explanation and justification by the analyst. Deviation for convenience, speed, or the whim of the analyst is not permissible for the regulatory analysis. A faster, simpler, more convenient method than the official one may be used as a screening procedure, but if this method produces results which show the product to be in violation of the law, check analysis must be made strictly by the official procedure. A manufacturer of a product cannot be expected to have his product or his freedom put into jeopardy by the use of an analytical procedure other than the *official* published procedure, if there is one.

If there is no official analytical method recognized for the particular product, then whatever analytical method is used must be shown to be valid for the product by appropriate recovery studies, analysis of synthetic mixtures, or other proper scientific validation studies.

The second part of the overall QA program is the national check sample program. The national check sample program forwards from Headquarters split samples as unknowns (known, however, to be quality assurance samples) with instructions for the field laboratory to treat these just as they would regulatory samples. The analyst in the laboratory who would normally receive the next incoming regulatory sample is to be assigned the quality assurance sample. The QA samples are not to be assigned in each instance to the laboratory experts. When the results are returned to Headquarters one of the things we check is to see that the same analyst does not repeatedly analyze the QA samples. On several occasions, we have tried to disguise the fact that these were not bona fide regulatory samples but rather quality assurance samples. Within minutes after arrival the truth is out and we still have not succeeded in hiding the true nature of quality assurance samples. The documentation accompanying a true regulatory sample is so extensive and definitive that it is practically impossible to provide properly disguised documentation.

Since we wish to test only one variable, that is the quality of the laboratory operation, the analytical method to be used is usually designated, and this is an official procedure wherever possible. We do not wish to check the method itself, although on occasion we learn a lot about the method from the results obtained. Occasionally we do allow the participating laboratory to use whatever analytical method it wishes in order to evaluate the laboratory's choice of methods. The samples provided may be a drug sample prepared to our specifications by a university laboratory, a commercial drug sample, a food sample with a naturally incurred pesticide or industrial chemical or sample with a spiking solution to be added, a sample to be analyzed for mycotoxins, etc.

As soon as the results are received from a laboratory, they are quickly reviewed for any obvious deficiencies. If such deficiencies are found, the laboratory is immediately communicated with either by phone or memo in an attempt to discover the reason for the deficiency. Additional analysis is sometimes called for. After the results are received from all the participating laboratories, a statistical analysis is made. We have made an arbitrary decision that any result falling beyond ±2 SD should be looked at carefully for possible areas of concern. A final report, together with the statistical study, outlining problems encountered and their solutions is then sent to all laboratories. Laboratory identifications are all coded. In order to give top management of the district in which the participating laboratory is located a quick picture of where their laboratory stands in relation to all the others, a bar graph is part of each report.

The third part of the overall QA program is the quality assurance audit visit. The quality assurance audit visit to the field laboratory checks on the data recorded as per the laboratory's internal quality assurance program and discusses the results of the national check samples. A representative number of analytical worksheets covering a variety of products, both domestic and import, and samples, both violative and in-compliance, are requested to be pulled from the files. These are reviewed by the visiting auditor, and then any deficiencies found are discussed with the laboratory supervisors and other laboratory management. An exit interview is held with top management of the field installation during which both the strengths and weaknesses of the laboratory operation are discussed. Recommendations for improvements are made.

In my previous statement concerning the two halves of the analytical equation—the measurement instrument and the human analyst—we believe now that the analyst is actually more than 50% of the equation. The problems found today in a regulatory laboratory demand for solution analytical methods so sophisticated, so demanding of a high order of skill and expertise, that the analyst performance has displaced the method itself as the major contribution to the variability in analytical results. Dr. Harold Egan, the Government chemist of the United Kingdom, reported in October 1975, during a symposium in London, England, on food analysis that the number of samples requiring his intervention as a referee laboratory has declined from about 120 per year at the turn of the century to the present level of about three a year. Considerable credit for this situation is given the open publication of analytical methods and their standardization through collaborative experimental study as in the AOAC procedure. Dr. Egan goes on to state, "In several major statutes in Britain, provision is made not for a referee *method* of analysis but for a referee *analyst* and in most instances the referee has a completely free choice of the method of analysis which he uses". It is the analyst who actually requires the greatest amount of attention in quality assurance programs.

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.