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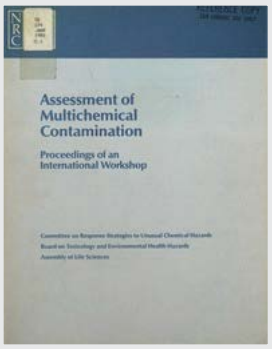

Assessment of Multichemical Contamination: Proceedings of an International Workshop

DETAILS

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Assessment of Multichemical Contamination

Proceedings of an International Workshop

Milan, Italy
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B. Paccagnella, *Chairman*
S. Murphy, *Cochairman*

Committee on Response Strategies to Unusual Chemical Hazards
Board on Toxicology and Environmental Health Hazards
Assembly of Life Sciences
National Research Council

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This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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PREFACE

In 1977, the National Academy of Sciences-National Research Council (NAS-NRC) was invited by the Italian government to join in a collaborative effort to investigate the effects of area-wide chemical contamination at Seveso, Italy. The contamination was the result of an explosion of a reaction vessel containing highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, commonly known as "dioxin"), which produced a cloud of chemical that was carried southward by the wind, exposing humans, animals, and plant life for several kilometers. The NAS-NRC sent a team of U.S. scientists to visit Italy and to determine with Italian officials the needs and opportunities for cooperative study. The NAS-NRC team recommended the development of a continuing relationship of U.S. and Italian scientists for the purposes of exchanging scientific and technical information, fostering the conduct of complementary research, organizing workshops and conferences to examine the impacts on health and the environment, and assisting in the coordination of exchange of scientists engaged in the analysis of this accident.

The NAS-NRC formally structured its involvement in this collaborative venture by establishing the Committee for Binational Cooperative Study of Exposure to TCDD, which was later renamed the Committee on Response Strategies to Unusual Chemical Hazards. The committee's terms of reference were

twofold: first, the development of guidelines that might be used to implement a worldwide mechanism for guiding biomedical researchers at the scene of accidents similar to that at Seveso (thereby ensuring the most comprehensive collection of scientific information in a timely manner) and second, the evaluation--in cooperation with Italian counterpart scientists--of newer health data from the Seveso accident and the design of future studies.

One of the committee's first undertakings was a workshop, held in March 1980 at the National Academy of Sciences, on Plans for Clinical and Epidemiological Follow-Up After Area-Wide Chemical Contamination. The Proceedings from that workshop have been printed recently by the National Academy Press. During that workshop, the U.S. committee and Italian scientists met to discuss plans for a second workshop to be held in Italy in 1981. It was agreed that the second workshop would serve as a conceptual framework for the development and refinement of investigative approaches to the study and prediction of the health impacts of multichemical exposures. Thereupon, in April 1981, a contingent of international scientists met in Milan, Italy, to participate in the International Workshop on the Assessment of Multichemical Contamination. Specifically, it was the intent of the workshop to elucidate the analytic, environmental, and toxicologic problems associated with chemical mixtures, to describe state-of-the-art investigational procedures, and to

advance concepts and approaches for the understanding of multichemical interactions influencing chronic risks to human health. This volume contains the papers that served as a basis for dicussions at that conference. The papers are grouped into three major categories: Identifcation of Analytic Issues, Environmental Interactions, and Toxicological Interactions of Mixtures in Humans and Laboratory Models.

PART I: IDENTIFICATION OF ANALYTIC ISSUES

Environmental Sampling Approaches and Procedures: A U.S. Perspective

Robert W. Risebrough, Brock W. de Lappe, and Wayman Walker II[1]

Within the United States, there is no consensus on the most appropriate procedures for the environmental monitoring of pollutant chemicals. This is equally true for programs that address problems associated with multichemical contamination and programs that address a single pollutant of particular concern. It is therefore difficult, if not impossible, to present in this paper a description of the U.S. perspective.

At the federal level, there are a number of monitoring programs for synthetic chemicals undertaken by several agencies, including the U.S. Fish and Wildlife Service, the Food and Drug Administration, the Environmental Protection Agency, and the National Oceanic and Atmospheric Administration. Frequently, there are parallel programs at the state level. Furthermore, perhaps more than in any other country, the private component has been actively involved in projects that assess the impact of pollutant chemicals upon human health, wildlife, and the integrity of the environment.

Although the rationale and the approaches may differ widely from program to program, there is a common element in that all

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have been developed in response to public concern over problems arising from the presence of synthetic chemicals in the environment. Almost always these are present as complex mixtures. Recent spectacular improvements in analytical methods have greatly increased the number of components within these mixtures that are detected. By providing us with a very different kind of data set, the new methods are forcing us to reexamine the concepts used over the past decade in our approach to environmental monitoring. Moreover, the accumulation of more than a decade's worth of data from past programs now permits an assessment of the extent to which these have fulfilled their mandates. The present symposium gives us, therefore, an opportunity to redefine some of the problems with a view toward developing new concepts and approaches.

Of necessity, we have prepared this paper on the basis of our own background and experience; we present, therefore, only one U.S. perspective on the problems of multichemical contamination. Our primarily ecological view acknowledges that man is the dominant member of the ecosystem. Moreover, it assumes a subtle, but important, difference between approaches that would protect human health and those that would protect other species and the integrity of the environment. In protecting wildlife we wish to maintain healthy populations. Mutations, birth defects, and cancers would not, except in extreme cases, affect the viability of a population. In

protecting human health, however, we specifically wish to prevent such effects. Separate approaches to these problems are not mutually exclusive; instead, they are, to a very large extent, inclusive.

Theoretically, within an ecological perspective we attempt to look at the broad picture. In this context we suggest that some of the solutions implemented a decade ago, in response to the problems as they were then conceived, have now produced another kind of problem. For example, many thousands of measurements of trace elements in environmental samples are being undertaken in various pollutant-monitoring programs without determining or assessing the magnitude of the natural fluxes of these elements in the local environment. Frequently the anthropogenic component of such fluxes is minor, yet the trace elements are usually referred to as "pollutants," suggesting a more massive perturbation of environmental processes by man than is actually the case. Such measurements waste considerable sums of money without necessarily being responsive to the need to provide public protection.

Similarly, there are programs involving many thousands of measurements of synthetic organochlorine compounds, although the majority of these pollutants are now ubiquitously distributed so that their presence in the environment is no longer news. Invariably, only the pollutant compounds already known and readily identifiable are reported, even when the

samples analyzed may contain many other components. But the existing data management systems almost always ignore the unidentified compounds. New approaches to environmental monitoring, therefore, should attempt to look at the complete picture, rather than only at those peaks on the chromatograms produced by known pollutants.

Our capability to detect an increasing number of synthetic organics in environmental samples has not been followed by an increase in our ability to assess their long-term significance. Unless there are biological effects, demonstrable now or in the future, monitoring programs will consist of little more than exercises in analytical chemistry and data reporting. Our inability to detect current biological effects is hardly proof, however, that they are not occurring. In developing new concepts in monitoring, we might consider methods for the detection of biological changes produced in response to a change in the chemical environment.

The past decade has witnessed a series of crises arising when a problem chemical has been detected in a local environment. How can we avert these crises, or at least anticipate them? Superimposed upon the other considerations, therefore, is the necessity to promote long-term protection by anticipating future problems, rather than merely reacting to the latest crisis. Using the past as a guide for the future, we shall present several case histories, reviewing how the

chemicals currently recognized as problems were first detected in the environment.

THE DETECTION OF BIOLOGICAL CHANGE

Since so many variables affect the health of individual members of both human and wildlife populations, an effect produced by a new synthetic chemical in the environment, or by an interaction of several synthetic chemicals, might not be distinguished above the background for many years. Relatively little attention is now being paid to the problems of detecting such biological change in the environment at an early stage. Perhaps a future workshop will consider the problems of detection as well as the measurement of biological effects associated with changes in the chemical environment. Few such changes will be as dramatic as the rapid disappearance of many forms of aquatic life from freshwater environments of northeastern North America and southern Scandinavia, which are resulting from acid rain (Dochinger and Seliga, 1976; Hutchinson and Habas, 1980). These gross perturbations will undoubtedly be followed by more subtle effects, resulting from the rapid leaching of many minerals from the soil cover.

The thinning of eggshells of a number of bird species is a further example of a widespread biological change that has been exceptionally well-documented. The phenomenon appeared abruptly, on a global scale, in the years immediately following

1945, providing evidence of a significant alteration at that time. It was not detected, however, for 20 years (Hickey and Anderson, 1968; Ratcliffe, 1967). Such a totally unanticipated outcome would never have been included in any model designed to predict the environmental effects of a chemical. We wonder how many other still undetected effects have occurred.

Since some wildlife populations accumulate exceptionally high levels of nonpolar synthetic organic pollutants, abnormalities observed in these populations provide an indication of possible future hazard to humans. Seals and sea lions inhabiting coastal areas are among the species known to accumulate high levels of organochlorines. In recent years four very different kinds of reproductive abnormalities have been documented. On the California coast premature births have occurred in populations of the California sea lion (Zalophus californianus), Steller sea lions (Eumetopias jubatus), and harbor seals (Phoca vitulina)(DeLong et al., 1973; Gilmartin et al., 1976; Risebrough et al., 1980a). In Puget Sound, there have been birth defects in the local population of harbor seals (Arndt, 1973). In the Baltic, a low level of productivity in ringed seals (Pusa hispida) has resulted from a high incidence of pathological changes of the uterus among females of reproductive age (Helle et al., 1976a, 1976b). In the western portion of the Wadden Sea, pup production has been below normal, and initial juvenile mortality has increased in

the local population of harbor seals (Reijnders, 1976, 1978). Cause-effect relationships remain obscure (Risebrough, 1979; Risebrough et al., 1980a), yet these problems merit detailed investigations.

In the early 1970s, a number of birth defects occurred in colonies of fish-eating birds in Long Island Sound (Hays and Risebrough, 1972) and in Lake Ontario (Gilbertson et al., 1976), suggesting the presence in local environments of chlorinated dioxins or similar teratogens (Bowes et al., 1973). Recent improvements in analytical methods now permit the measurement in environmental samples of 2,3,7,8-tetrachlorodibenzo-p-dioxin. This compound has recently been detected in archived eggs of herring gulls (Larus argentatus) at levels sufficient to account for the observed abnormalities in Lake Ontario (D.J. Hallett, R.J. Norstrom, personal communication).

We should, therefore, continuously monitor wildlife populations to detect biological and associated chemical changes that might have implications for human health. But there is no standard method. Moreover, wildlife populations appear to be of little value in the detection of environmental carcinogens; high incidence of mortality resulting from such a cause would be extremely difficult to detect.

Environmental sampling is one approach toward finding the causes of the increased incidence of human cancers in certain

areas that appear to be environmentally related. The development of sensitive biological tests for the presence of mutagens that are also potential carcinogens (Ames et al., 1975) permits the examination of extracts of air, water, or sentinel organisms from these areas. Since the amounts of material are not limiting, sample extracts that could provide a positive response to the test could be fractionated and studied in detail to determine which chemicals are the causative factors.

TOXIC CHEMICALS AND ENVIRONMENTAL CRISES: THE PAST DECADE

In revising our strategies for environmental sampling and monitoring, we hope primarily to avert or at least minimize the effect of future crises resulting from the unanticipated presence of toxic chemicals in the environment. We should, therefore, consider examples from the recent past in developing such strategies.

The Chlorinated Dibenzodioxins

The chlorinated dibenzodioxins, particularly 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), are of particular relevance in northern Italy, as a consequence of the explosion in Seveso that released significant quantities of TCDD into the local environment. TCDD is now recognized as an environmental problem chemical in Lake Ontario and in an area of Lake Huron, having entered the lakes as a component of industrial wastes and byproducts. Extensive contamination of the food webs of

the lakes has occurred over the past decade (D.J. Hallett and R.J. Norstrom, personal communication).

The first clues to the presence of this compound in Lake Ontario included a very high incidence of embryonic death in fish-eating birds in the late 1960s and early 1970s (Fox et al., 1975; Gilbertson, 1974; Gilbertson and Hale, 1974), as well as an apparently high incidence of birth defects (Gilbertson et al., 1976). These had prompted an earlier search for the presence of TCDD (Bowes et al., 1973), which has only more recently been detected through more sophisticated analytical techniques (D.J. Hallett and R.J. Norstrom, personal communication).

Because of its very low levels in environmental samples, TCDD has not been detected in routine analyses of mixtures of synthetic organic compounds. Rather, it has been detected only as a result of specific searches where its presence was suspected. These suspicions arose from a knowledge that processes involving 2,4,5-trichlorophenol are likely to produce this dioxin, and from the biological observations of a high incidence of embryonic death and an apparently high incidence of birth defects. Similar observations of biological abnormalities in the future might indicate the presence of other teratogens in the environment. Moreover, biological indicators would probably provide a more sensitive index than would chemical methods.

The DDT Compounds

A U.S. policy decision to prohibit any contamination of the milk supply by dichlorodiphenyltrichloroethane (DDT) and its derivatives probably comprised the factor that first led to the recognition that the DDT compounds had become significant environmental pollutants. In response to the need to enforce this tolerance level, and those imposed for other foods, analytical methods, particularly electron-capture gas chromatography, developed rapidly in the 1960s. The use of such methods in the monitoring of foods therefore provided the data that first indicated the widespread occurrence of p,p'-dichlorodiphenyl-2,2-dichloroethylene (DDE) in the environment.

The first of a series of biological changes that were later associated with DDE was the population reduction and local extirpation of the peregrine falcon (Falco peregrinus) in Britain, areas of western Europe, and eastern North America (Hickey, 1969; Ratcliffe, 1980). Initial observations of the local disappearance of the species were made in the mid-1960s. Comparable observations of a number of other species quickly followed, particularly with the discovery of eggshell thinning. Several research papers subsequently demonstrated that most if not all of the shell thinning, as well as the population declines, can be attributed to DDE (reviewed by U.S. Environmental Protection Agency, 1975).

Thus, a need to protect the food supply from "DDT" led to the discovery that a derivative compound, p,p'-DDE, was accumulating in local environments. Biological observations indicated the existence of widespread deleterious effects, which were only later shown to be caused by DDE.

The Polychlorinated Biphenyls

The development of electron-capture gas chromatography also led to the discovery that polychlorinated biphenyls (PCBs) were widespread environmental contaminants. These substances first appeared as unidentified peaks on gas chromatograms during analyses of environmental samples for DDT compounds. The unknown pollutants were detected at high levels in species showing patterns of local population decline, including the white-tailed eagle (Haliaetus albicilla) in Sweden and the peregrine falcon in western North America. Laboratory analyses, based on extensive programs of biological sampling undertaken in Sweden, the Netherlands, and North America, indicated that PCBs were just as widely distributed as DDT compounds (Jensen et al., 1969; Koeman et al., 1969; Risebrough et al., 1968). The Yusho epidemic, which began in 1968, associated with PCBs as well as the contaminant chlorinated dibenzofurans (Kuratsune et al., 1976), demonstrated the human health significance of these compounds and prompted measures to phase out their use. A systematic effort to identify principal unknown peaks on gas chromatograms, before it was known that

they represented any particular hazard, resulted therefore in a significant environmental discovery.

Dieldrin and Related Pesticides

Several countries, including the United States, have discontinued or severely restricted the use of dieldrin and other pesticides, including heptachlor, the chlordane compounds, and endrin. Primarily, they are suspected of being potential human carcinogens. Their widespread occurrence in the environment, as shown in a number of monitoring programs, indicated that they had sufficient environmental stability to reach humans through the food webs. The history of this class of biocides provides the justification for a monitoring network that is repetitive over both time and space. This approach establishes a data bank that presents a picture of the geographical distribution of a particular biocidal compound in the environment, as well as changes in levels with time.

Mirex

Mirex, previously used in the southeastern United States to contol fire ants and now extensively used in tropical countries to control leaf-cutting ants, is a suspected human carcinogen. On this basis, this country has discontinued its use. Monitoring programs that provided data indicating both its mobility and stability in the environment played a key role in the decision.

Mirex is also among the many organochlorine contaminants of

the Lake Ontario ecosystem. A program for multichemical pollutant analysis of environmental samples first identified it in fish (Kaiser, 1974). Subsequent studies revealed that mirex is a widespread contaminant throughout the Lake Ontario food webs (Hallett et al., 1976; Norstrom et al., 1980), originating from a factory discharge into the Niagara River. Mirex also turned up in marine mammal tissue in the Netherlands (Ten Noever de Brauw et al., 1973), where it had been used under another name as a fire retardant. Its detection was again the result of multichemical analyses of environmental samples.

Kepone

The contamination by kepone of the James River Estuary in the state of Virginia has led to severe restrictions on both commercial and recreational fishing in the area, causing extensive economic damage. This time, a monitoring program was not responsible for the detection of the presence of kepone in the local environment. Rather, workers at the manufacturing plant in Hopewell, Virginia, began to show symptoms of neurological illness, which were attributed in 1975 to kepone contamination. A preliminary survey revealed its widespread distribution in the local environment, as a result of factory discharge of kepone-contaminated waste into a local watershed (Huggett and Bender, 1980; Huggett et al., 1980).

Summary

There are a number of other examples, including the environmental contamination in Michigan by polybrominated

biphenyls (PBBs) manufactured as fire retardants, but these case histories are sufficient to provide guidelines for revising our monitoring and sampling strategies:

1. systematic efforts to identify unknown peaks on gas chromatograms have played a major role in the detection of problem chemicals in the environment;

2. worthwhile results have been produced by programs that "go out and see" what is in the environment;

3. biological observations in many cases have provided the first indication of the presence of problem chemicals in the environment; and

4. important data on one contaminant have been obtained from programs designed to monitor other groups of compounds.

Identification of unknowns on gas chromatograms and determination of the environmental distribution of the corresponding pollutants should therefore be a part of future monitoring strategies. Furthermore, our approach should look at the broadest possible spectrum of the pollutant chemicals detected, whether or not all are currently identified.

THE DATA BASE

Data Collection

Over the past 10 years or more, U.S. monitoring programs have examined the distribution of synthetic organic compounds, principally pesticides, in various environmental components including soils (Carey, 1979; Carey et al., 1978, 1979); total diet samples, comprising the "market basket" survey (Johnson

and Manske, 1976); the wing muscles of waterfowl (White, 1979b; White and Heath, 1976); freshwater fish (Veith et al., 1979); starlings (Sturnus vulgaris)(White, 1979a); tissues of bald eagles (Haliaetus leucocephalus) (Kaiser et al., 1980); and coastal and estuarine mollusks, principally Mytilus (Butler, 1973; Butler et al., 1978; Goldberg et al., 1978). The results of these programs and others of shorter duration have been the subject of many research papers and reports, so that a significant paper glut has developed. Attempts to computerize data within some programs have begun, but relatively little progress has been made in rapid retrieval of information from a data base common to more than one program.

So far these programs have reported only the identified compounds. The results on the printed page rarely provide an indication of the relative abundance of unidentified compounds.

Within our own perspective we shall comment principally on the Mussel Watch Program, which has continued the earlier efforts of Philip Butler (Butler, 1973; Butler et al., 1978) to monitor a variety of pollutants in coastal waters, using mussels (Mytilus sp.) and other bivalves as indicator organisms. In 1976, the first year of this program, only the DDT and PCB compounds among the organochlorines were reported (Goldberg et al., 1978). Studies the second year revealed chlordane and hexachlorocyclohexane compounds, hexachlorobenzene, dieldrin, and endrin in many of the samples. During the third year, analyses were conducted by

capillary column electron capture, as well as by flame-ionization-detector gas chromatography. Adoption of this method has caused a crisis in data management, which will extend to the other monitoring programs as the capillary techniques are adopted as routine. Figures 1 and 2 illustrate the nature of this problem; they depict the aromatic fraction of extracts of mussels (Mytilus edulis) obtained from Boston Harbor and oysters (Crassostrea virginica) from Cape Charles, Virginia. The majority of the peaks represent unknown substances; the three other fractions obtained in the analytical separation also contain many unknown pollutants detected by capillary column electron-capture chromatography. Each analysis may therefore yield information on more than 100 individual compounds. In reporting data on only the few identified, we ignore significant quantities of information; the number of individual compounds detected, however, imposes major constraints in the storage and reporting of this information.

Data Management

A monitoring strategy for the 1980s cannot be implemented without consideration of a data management system. In part, the U.S. Mussel Watch Program is tackling the problem in the following way: Each of the resolved peaks is assigned a Kovats index for the column type employed. This index, developed in flame-ionization-detector gas chromatography, is based on the relative emergence time of the n-alkanes. Thus, n-C_{17} is

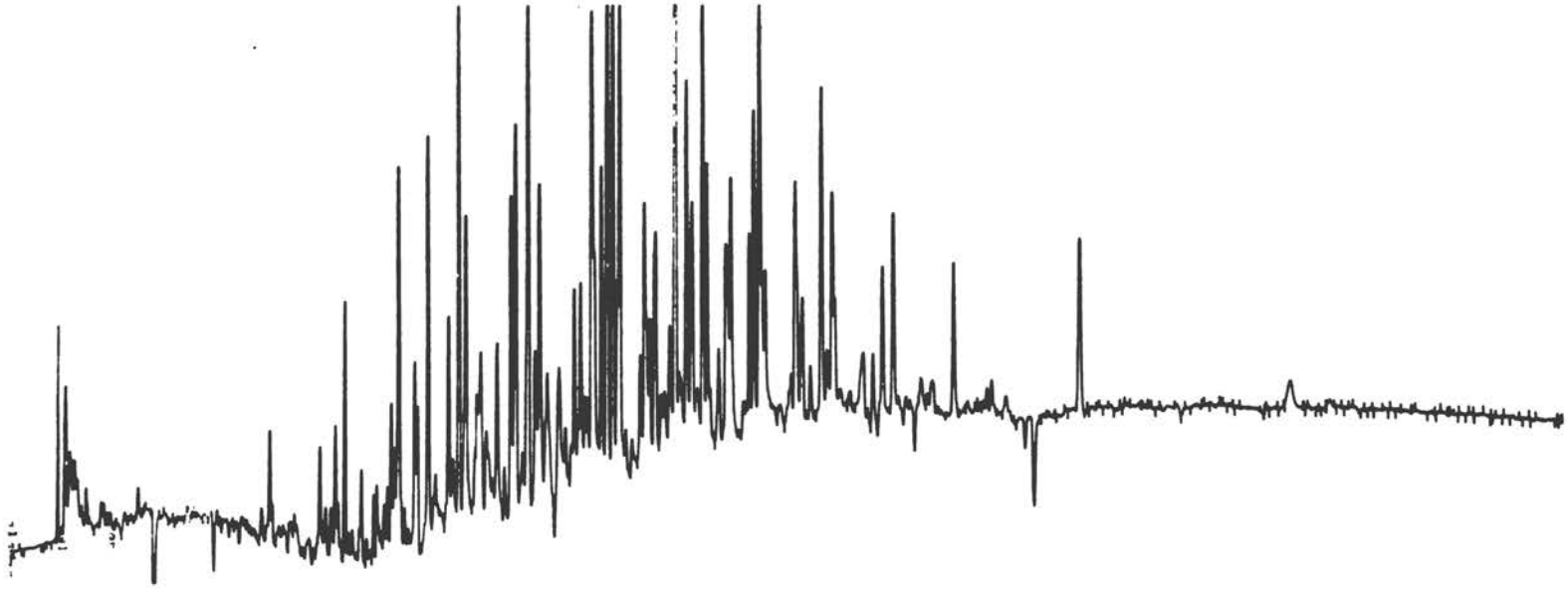

FIGURE 1. Electron-capture chromatogram of aromatic (F_2) fraction, extract of mussels from Boston Harbor, 25 September 1978, National Mussel Watch Program, Bodega Marine Laboratory. 30 m SE-54 fused silica column, Carlo Erba 2150 gas chromatograph. This fraction is one of four obtained in the separation procedure. More than half of the peaks represent currently unidentified compounds.

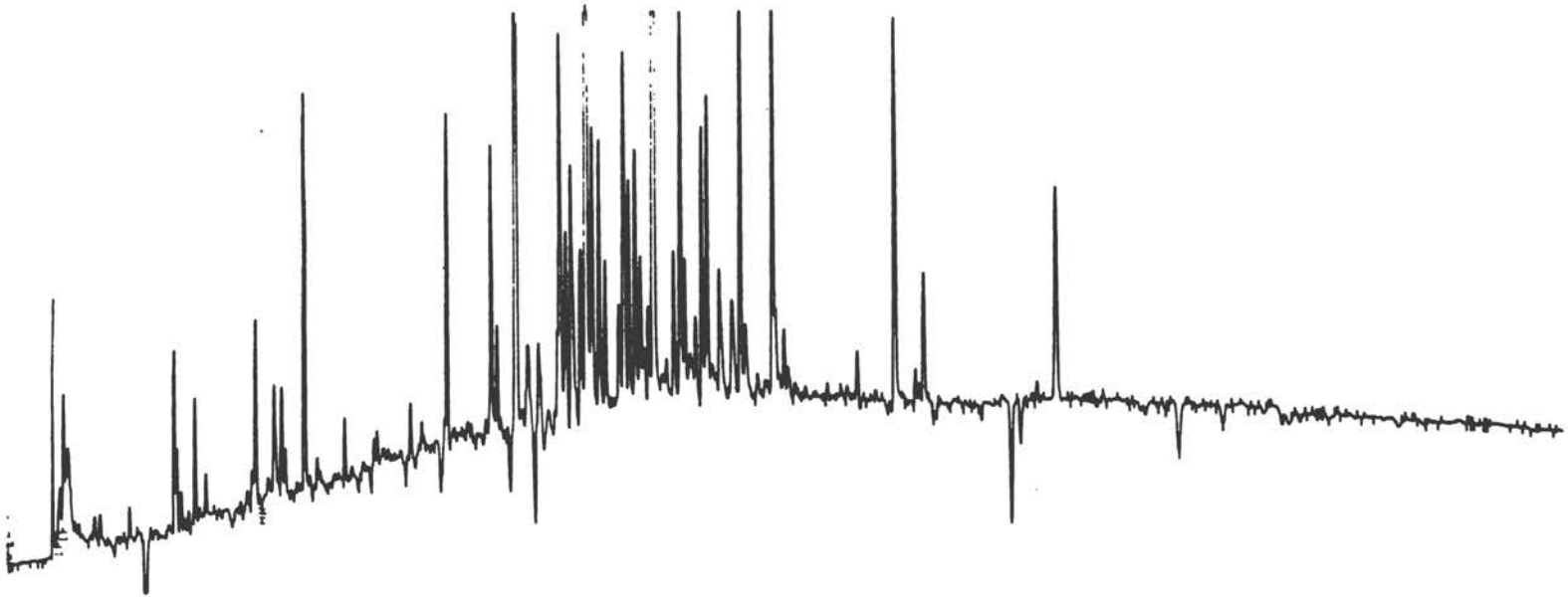

FIGURE 2. Aromatic (F_2) fraction, extract of oysters, Crassostrea virginica, from Cape Charles, Virginia, 21 October 1978, National Mussel Watch Program, Bodega Marine Laboratory. 30 m SE-54 fused silica column, Carlo Erba 2150 gas chromatograph. The majority of the peaks represent currently unidentified compounds.

assigned a Kovats index of 1700, n-C_{18} a Kovats index of 1800. On the SE-30 column, pristane emerges at one-tenth of the distance between the two alkanes and is thereby assigned a Kovats index of 1710 for this column type. Coinjection of alkanes and the various synthetic organics permits assignment of a Kovats index for the latter, which can then be used as a basis for the assignment of indices to unidentified peaks on the electron-capture chromatograms. A computer program permits the calculation of Kovats indices based on retention times and of an estimate of concentration from the integrated areas, frequently based on the response factor of an internal standard. Labels are assigned as compounds are identified by gas chromatography/mass spectrometry or other techniques.

Further developments in this area will no doubt include storing all relevant sample information, including collection data, the derived data produced by the current generation of gas chromatographic outputs, as well as the gas chromatographic signal. Additional expansion of the data base further indicates that the concept of producing tabulations of all data on paper is becoming obsolete. Computerization of the data base permits retrieval of only those data needed for a specific purpose, making hardcopy outputs only as required. Thus, the data management approaches of the 1970s will not apply to the 1980s.

MONITORING STRATEGIES: SOME GENERAL CONSIDERATIONS

Monitoring programs designed to address specific questions, and to obtain a data set relevant to those questions, are more likely to be successful than those that gather data in a "look and see" approach to environmental surveillance (National Academy of Sciences, 1980). The U.S. national monitoring programs generally address the distribution of persistent pesticide residues in environmental components, including soil, river waters, freshwater fish, terrestrial birds, the wing muscles of waterfowl, and the tissues of the bald eagle, an endangered species that is at the top of a food web that includes fish. To date, the Mussel Watch Program has operated more in a research mode than in a monitoring mode, primarily consisting of a "look and see" operation. The data obtained on pollutant levels in bivalves in the coastal zone, however, should help us to implement long-term policies for protection and management.

The Mussel Watch Program shares with other national programs a strategy that dictates that measurements be repeated over both space and time. Spatial measurements not only assist in pinpointing input sources of pollutants, but they also assist in the interpretation of the significance of local pollution levels. Thus, the existence of a data set from San Francisco Bay, which can be compared to data from other areas of the California coast as well as the rest of the country and the world, is useful in examining the hypothesis that one or a

combination of pollutants might be responsible for lesions observed in a local species of fish. If other bays and estuaries have comparable levels of particular pollutants and the local fish exhibit no such lesions, this would suggest that these pollutants do not play a major role in causing the lesions.

Measurements over time permit determinations of increases or decreases in environmental levels in response to increased input or to administrative or other actions that decrease environmental input. Thus, the Mussel Watch Program has determined that levels of DDE and PCBs in the California coastal environment have declined by up to an order of magnitude over the past decade (Risebrough et al., 1980b).

The expansion of the data set, and the impossibility of managing these data on paper, emphasize the necessity for a greater coordination among the existing programs. If a new problem chemical is identified in the environment, it would be advantageous to search an extensive data file to determine if it might be one of the previously observed "unknowns."

In this brief paper, we have not mentioned sampling strategy and the problems of reducing sampling variance and of ensuring that samples taken are a good representation of the environmental component being monitored. The approaches taken and progress made in the Mussel Watch Program have been presented in the Proceedings of the Barcelona Mussel Watch Workshop (National Academy of Sciences, 1980) and in a summary

of the Mussel Watch Program in California (Risebrough et al., 1980b).

The high economic cost incurred through the loss of food resources and employment as a result of toxic chemical contamination over the past decade would alone justify continuing surveillance programs. Furthermore, there are the unmeasureable risks to human health and the potential damage to wildlife populations, which cannot be quantified. It would appear, however, that a greater level of coordination among the participating institutions is required to achieve a successful solution to the problems we now face.

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Comprehensiveness and Separation of Samples

Milos Novotny[1]

A multitude of toxic substances produced during various technological processes dictates a continuous development of effective analytical methods for assessing potential health hazards. In such a way, modern analytical chemistry becomes involved in the processes of recognition, evaluation, and control of chemical hazards.

The analytical problems associated with chemical contamination of the environment vary from one case to another. Specific procedures can determine known toxic substances even in extremely complex sample matrices. Frequently, the necessary methodology will have impressive sensitivities and precision, often down to part-per-trillion levels. Very small amounts of selected pollutants must often be measured with high sensitivity and accuracy to assess the potential for personal exposure, bioaccumulation, metabolism, or the compound's persistence in the environment. Knowing what substances to look for simplifies the process, and some knowledge of the related industrial process can also be helpful in elucidation of any additional toxic substances,

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such as starting materials and by-products.

The release of toxic dioxins into the environment on various occasions and the subsequent human exposure (see Holmstedt, 1980, for a review) are examples of this type of situation. A chemist's solution will almost always involve highly selective instrumental techniques that "ignore" the remaining sample constituents. Though expensive, such techniques can provide adequate quantitative answers without extensive sample fractionation. The well-established mass-fragmentographic approach or the recently advocated mass spectrometry/mass spectrometry methods (Kondrat and Cooks, 1978) have this general capability or potential.

Multichemical contamination situations are significantly different. If the area of toxic hazard is not sufficiently defined, additional strains are placed on both sampling and analytical measurement procedures to ascertain comprehensiveness. Even the best available sample separation schemes and measurement methods are challenged when one endeavors to determine what environmentally important substances are contained in a sample of either a suspected health hazard or a proven toxicity.

Complications are numerous, as illustrated by the Love Canal story (Axelrod, 1980), among others, in which numerous industrial by-products in different quantities were deposited at one place over a period of time. In such instances, the original complexity can be magnified by various additional

reactions among the mixture components, which, in turn, may multiply the number of compounds to be included in analytical as well as toxicological considerations (e.g., various synergistic effects). This chain reaction is likely to continue due to weathering, oxidations, and interactions with the ecological system. Naturally, some detoxification processes may take place, but the opposite can also be true, forming more and/or other highly toxic substances.

Such a scenario is a nightmare for toxicologists, epidemiologists, and analytical chemists alike. One cannot accurately predict either the occurrence or the concentrations of the toxic substances. Even sample components present in lesser (or even trace) amounts cannot necessarily be labeled as toxicologically unimportant.

Therefore, although extensive toxicological screening and specialized tests will provide the most important information on the hazards of a given multichemical contamination source, one must simultaneously conduct a detailed chemical characterization, given the limitations of methodology and cost. This is a formidable task: the sample components may span a respectable range of molecular weights, polarities, and other chemical features. Certain biological effects could be derived from organic pollutants, toxic metals, or a combination of both. Different toxic substances can be present with a large dynamic range of concentration. If sufficiently simple, specific analytical screening methods for the compounds of

known toxicity can be undertaken for orientation purposes.

However, these procedures cannot substitute for a more comprehensive approach to sample characterization, which should include (a) a comprehensive extraction; (b) fractionation into different compound classes; (c) chromatographic separation and detection of the individual sample components; and (d) positive identification and quantitation of the environmentally important sample components within a specific concentration range. Such general analytical methods have a precedent in modern analysis of biological materials, in which samples of comparable molecular complexity are frequently encountered. Certain directions in environmental trace analysis, initiated several years ago, have similar methodological goals. The U.S. Environmental Protection Agency has been working on the so-called Master Analytical Scheme (Donaldson and Garrison, 1979; U.S. Environmental Protection Agency, 1981). Another approach, for sample screening for more than 100 pollutants in common water samples (U.S. Environmental Protection Agency, 1979), attempts to develop a general protocol for analytical survey and regulatory purposes. Although the Master Analytical Scheme has been criticized (Ehmann et al., 1979; Golton, 1979) for its potential problems, the reasons for a standard, unified approach are fully justified. In this paper I will evaluate, in general terms, some procedures that could lead to a comprehensive characterization of environmental mixtures pertaining to multichemical contamination. I will also discuss state-of-the-art analytical methods.

SAMPLE PREPARATION AND FRACTIONATION

Any successful screening for environmentally important compounds, in either qualitative or quantitative terms, must begin with adequate sample preparation. The analysis could cover a variety of materials, including contaminated soil or water, waste oils, plant materials, and animal tissue, so an adequate sample homogenization and exhaustive extraction must be ascertained first. Here, there are only a few general guidelines, since there are usually some variations from one sample to another.

Sample preparation strategy depends also on the roughly expected amounts of toxic substances. Extraction is a relatively straightforward task, if one deals with solid wastes or material directly from a chemical dump. Even in the vicinity of a major contamination site, the concentrations of pollutants will be at least in the part-per-million range. At much lower levels, from a few parts-per-million to parts-per-trillion (limits given by the current sensitivity of modern instrumentation), legitimate concerns arise about the efficiency of extraction and sample losses due to the clean-up procedures.

Solvent partition is generally recommended for recovery and concentration of organic pollutants from aqueous media, whereas the Soxhlet extraction is suitable for various pulverized materials. Solvents of a great eluting strength cover a wide range of organic compounds to be extracted. A few definite

studies concern extraction efficiency of different solvents, but methylene chloride seems to be generally favored as a solvent of adequate purity, low boiling point, and good extraction capability. Other solvents are occasionally used for specific purposes.

Alternative methods should exist for polar organic compounds, which are not readily extractable from aqueous media with organic solvents. During the development of new methods, the efficiency of extraction and other preliminary sample treatments are frequently checked with isotopically labeled compounds carried through the entire sample-preparation procedures. Obviously, the proper choice of internal standards may be crucial to overall accuracy. Ideally, there should be multiple standards for determining several different compounds. At best, this is an impractical proposition, and some compromises must be met in practice.

When toxic pollutants of interest are encountered in aqueous media, there are additional means of sample concentration. One can trap trace organics on various solid granular materials while pumping an appropriate amount of a contaminated sample through them. Thus, the substances of interest are collected on "accumulator columns" packed with activated carbon, inorganic adsorbents, polyurethane foams, porous organic polymers, and reversed-phase packings (see reviews in Garrison et al., 1979; Keith, 1976). Under suitable conditions, such materials strongly retain organic trace

components, which can later be recovered for further separation, characterization, or quantitative analysis. There are similar procedures for trapping relatively volatile compounds purged with a gas.

After accumulating sufficient sample amounts, one can analyze the content of the sampling tubes. Recovery procedures may involve either thermal desorption into a gas-chromatographic column, in case of volatile substances, or solvent extraction. Quantitative evaluation of the content of accumulator columns requires careful standardization (Novak et al., 1965; 1979).

In most cases of multichemical contamination, the mixtures of extracted organic compounds will be complex. Compound identification and/or reliable quantitation require chromatographic analyses at high resolution. However, even the best chromatographic systems will not provide complete separation of all possible mixture components and reliably determine the compounds of interest in complex sample matrices. Thus, there must be further fractionation of these extracts or otherwise preconcentrated mixtures.

Various classes of compounds can be obtained through an appropriate analytical fractionation scheme. Even selective enrichment is sometimes feasible if the compounds contain some unique structural features. This type of sample fractionation is sometimes synonymous with "sample clean-up," as in the removal of ballast materials during low-effiency chromatography

or solvent partition steps in any trace analysis of agriculturally important chemicals. Further examples of more or less selective fractionations involve the use of ion-exchange materials to retain acidic or basic substances, or "extraction" of volatile chemicals from nonvolatile matrices with a stream of gas and adsorption on porous polymers.

Relatively effective fractionation procedures are based on solvent partition schemes, which separate the mixtures according to acid-base properties and polarities (see Figure 1 for an example). This general approach was originally developed for fractionation of tobacco smoke, one of the most incredibly complex mixtures, but it can be adapted to other analytical problems. Each of the separated fractions is then subjected to detailed chromatographic investigation. Depending on relative representations of the components, which vary from one case to another, certain overlap among the fractions may occur, and further "clean-up" may be desirable. Alternative analytical schemes may be based on various forms of column chromatography (gels, ion-exchangers, or adsorbents), but some concerns exist about possible losses of various compounds on the columns.

The compounds of interest may cover a wide range of volatility and polarity. Consequently, highly efficient forms of both gas and liquid chromatography should be complementary rather than competitive approaches to a satisfactory analysis,

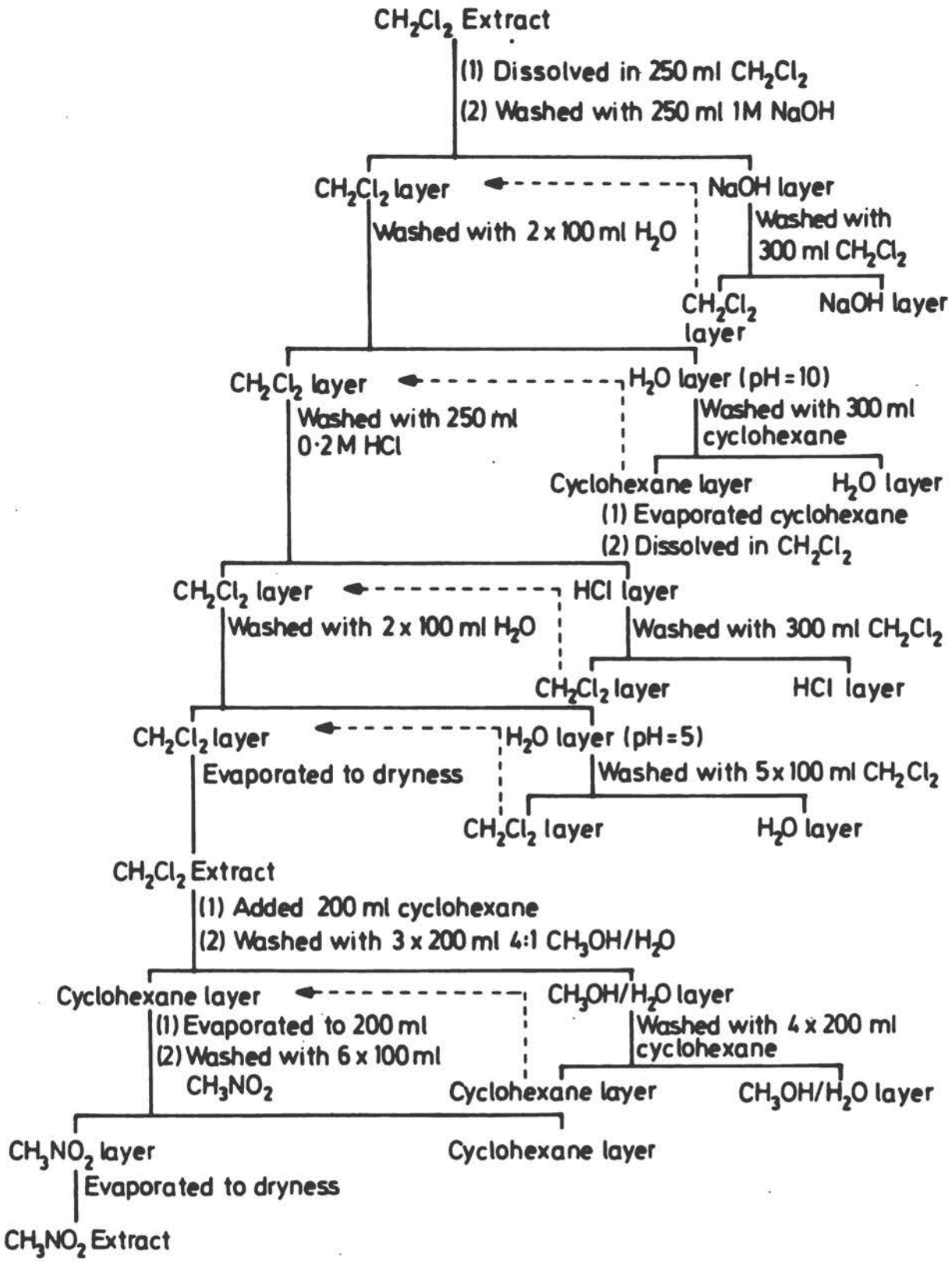

FIGURE 1. Solvent partition scheme for complex mixtures. (From Novotny et al., 1981. Reproduced with permission of IPC Science and Technology Press, Ltd.)

especially because only some 10 to 20% of the components in environmental extracts are sufficiently volatile and amenable to gas-chromatographic analysis.

For the sake of completeness, samples of a suspected or proven environmental hazard should also be screened for the presence or absence of certain toxic elements, such as heavy metals. Here, the necessary method for enrichment and analytical measurements is vastly different from the other approaches. The modern methods of simultaneous multi-element analysis are fully capable of determinations within a wide range of concentrations. Among them, inductively coupled plasma spectroscopy appears most prominent (for a review, see Dahlquist and Knoll, 1978). Direct sample analyses are often feasible; however, sample preconcentration is sometimes required for analysis (see reviews in Gould, 1968; Minczewski, 1967). When toxic elements are found at environmentally alarming levels through a multi-element atomic spectral technique, further studies are in order to elucidate the speciation aspects.

GAS-CHROMATOGRAPHIC ANALYSIS

Sample Volatility

Modern gas chromatography spans an impressive range of sample volatility. The advent of thin-film capillary columns and thermally stable stationary phases has enabled analysis of relatively large molecules in the gas phase, provided that they are stable enough themselves at column temperatures extending

up to 350°C. Under such circumstances, molecules as large as alkanes with carbon number over 50, certain triglycerides, and eight-ring polyaromatic compounds, can be successfully chromatographed. Figure 2 shows an example of a mixture of polycyclic aromatic molecules chromatographed on a thermally stable capillary column (Hirata et al., 1981).

Modern gas chromatography, therefore, can analyze numerous toxicologically important compounds, including many industrial and agricultural chemicals, chlorinated pesticides, dioxins, and various other, possibly carcinogenic compounds. The more polar types of these substances may, however, suffer from decomposition problems during gas chromatography due to their limited stability and undesirable interactions with the analytical systems. These problems are particularly evident with very low sample quantities. Then high-performance liquid chromatography (HPLC) would be a better analytical alternative, but the problems of universal detection and sensitivity still exist.

The volatility range of gas chromatography can be extended by chemical derivatization techniques in certain cases. Thus, many polar and nonvolatile compounds can be quantitatively transformed into derivatives amenable to gas chromatography, an approach well-established in biomedical analyses (see the relatively comprehensive reviews of different derivatization techniques in Drozd, 1975; Knapp, 1979). There are also a limited number of environmental applications of this general

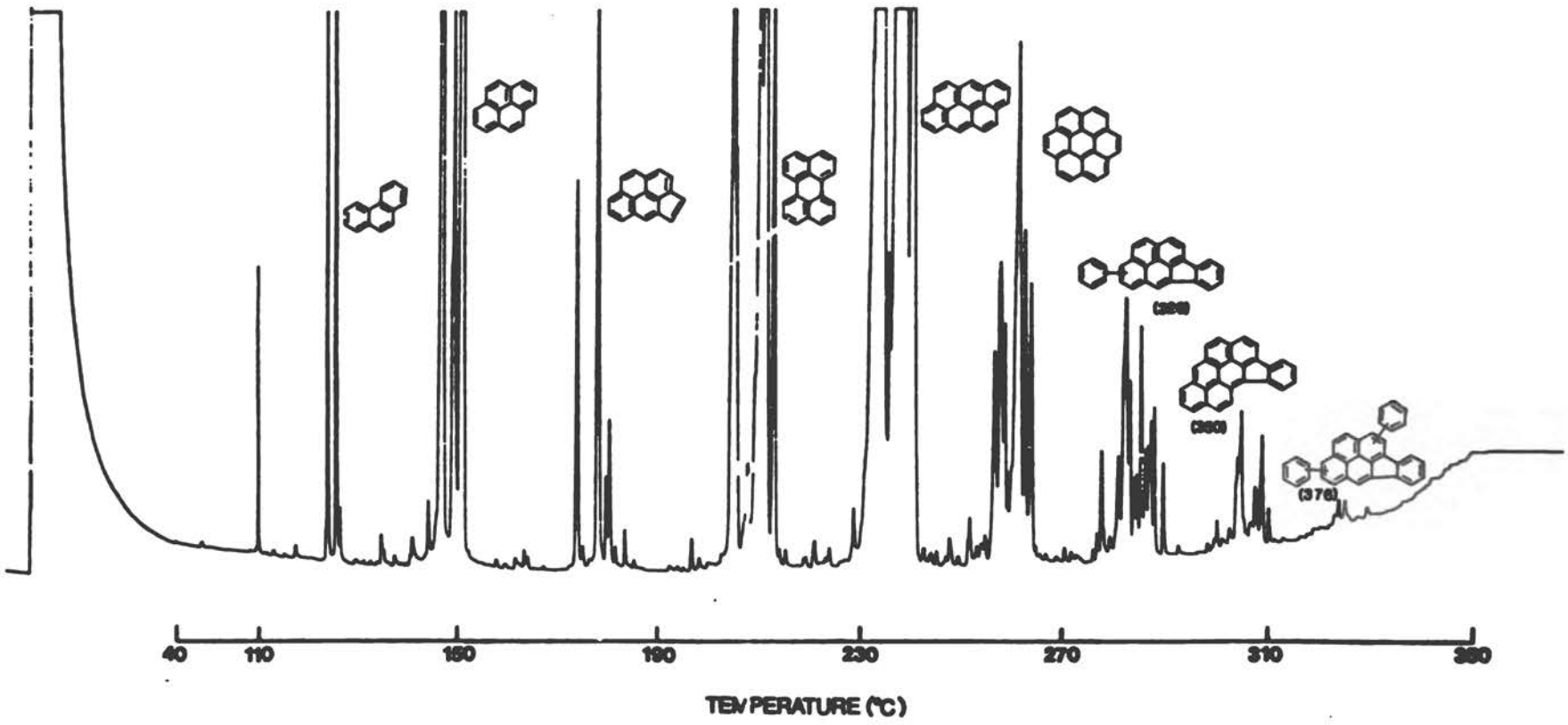

FIGURE 2. High-temperature trace analysis of polynuclear aromatic compounds extracted from carbon black. (From Hirata et al., 1981. Reproduced with permission of Elsevier Publishing Company)

approach, as in the alkylation of organic acids or silylation of phenolic compounds.

Chromatographic Resolution

Today, capillary columns are rapidly replacing packed columns for all but the most trivial gas-chromatographic separation problems in environmental analysis. This is due primarily to the high degree of complexity associated with environmentally important mixtures. It is usually necessary to separate the compounds of interest from background materials to identify them and ascertain their quantities at appropriate levels. Today's glass and fused-silica capillary columns can resolve hundreds of mixture components within a single chromatographic run, often without tedious and time-consuming clean-up procedures.

Another important feature of capillary gas chromatography is its remarkable resolving power for structurally similar compounds. Extremes of high resolution have been widely documented in this field with the separation of optical isomers or cis-trans and positional isomers. This is significant for environmental chemistry, as there are many cases in which different isomers of a basic structure can have vastly different biological properties, including toxicity.

Characterization of the mixtures associated with multichemical contamination is a perfect case for capillary gas chromatography. Many dangerous industrial pollutants, including polychlorinated biphenyls (PCBs), are already manufactured as complex mixtures, as demonstrated in Figure 3

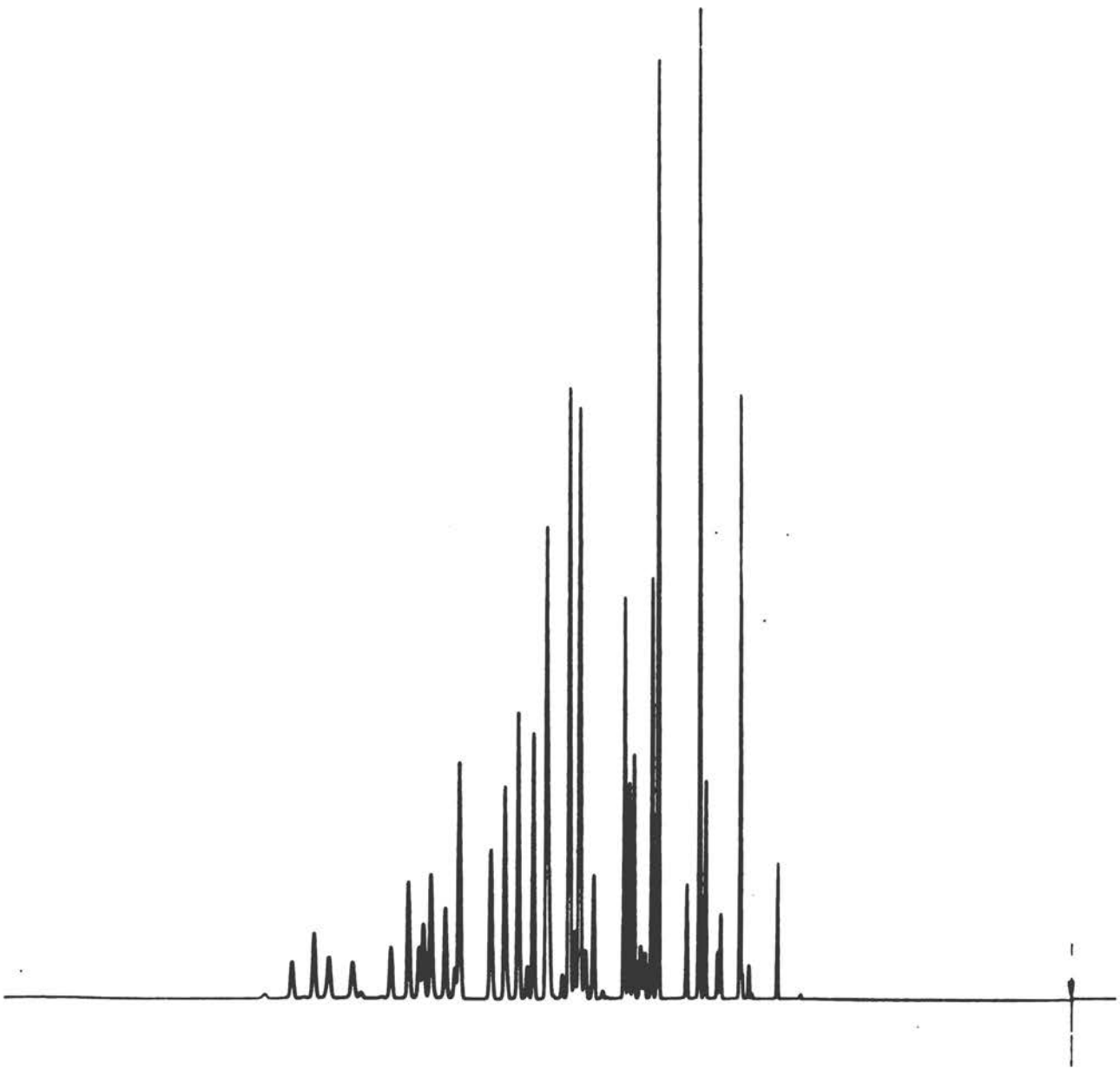

FIGURE 3. Gas chromatographic separation of isomeric polychlorinated biphenyls (Aroclor 1016) at 220° C. (From Schomburg et al., 1974. Reproduced with permission of Elsevier Publishing Company)

(Schomburg et al., 1974). Incidental blends of dangerous chemicals created at chemical dump sites also require the highest available component resolution. The term "chlorinated dioxins," for example, comprises some 75 related compounds, whereas 22 possible isomers already exist for tetrachlorodibenzo-p-dioxin (Holmstedt, 1980).

Detection Methods and Quantitative Determinations

Sensitivities of the commonly used detectors complement the operating concentration range of modern gas-chromatographic columns. Sensitivities at the low nanogram levels are very common, while some detectors reach levels even lower than picogram amounts.

To screen for toxicologically important compounds, as well as giving a broad characterization of a given sample, one should use the flame ionization detector in conjunction with a capillary column. This device provides universal detection of all organic compounds, within a given volatility range, at subnanogram sensitivity. Thus, within that range of volatility and sensitivity, no sample component should be missed during the initial screening. Resolution of the individual sample components from each other can be optimized at this stage, while further investigations by combined gas chromatography/ mass spectrometry are in order.

In the past, the major incentive for developing gas-chromatographic selective detectors has involved overcoming the problems of sample complexity with packed columns. These

detectors are "blind" to compounds in a mixture that do not possess certain unique structural features, i.e., chromophores or heteroatoms. But these detectors and capillary columns significantly enhance each others' capabilities (see Table 1 for the sensitivities and some other features of the most common selective detectors).

Since parallel use of the flame ionization detector and certain selective detectors is now widespread, perhaps similar equipment should be used in sample screening and characterization for multichemical contamination cases. Many environmentally important compounds have structural features that are compatible with these detectors. Figure 4 (Grob, 1975) shows an example in which the flame ionization and electron capture detectors are used as a means of complementary detection: the electron capture recording suggests the presence of trace amounts of PCBs, while the flame ionization detector indicates a complex mixture (as might be expected with this sample type).

Various techniques in gas chromatography/mass spectrometry provide the utmost in detection selectivity. Their utilization may range from the common spectral scanning mode for compound identification to measurements of preselected ions arising from suspected pollutants. The commonly used techniques of mass chromatography provide valuable information on the presence and the levels of different compound classes. This paper will not discuss these methods in detail, but will emphasize two major

TABLE 1

Properties of Some Gas Chromatography Selective Detectors

Detector	Selectivity mode	Approximate sensitivity (g)
Electron capture	Affinity to low-energy electrons	10^{-13} - 10^{-14}
Thermionic	Nitrogen	10^{-12}
	Phosphorus	10^{-13}
Flame photometric	Sulfur	10^{-9}
	Phosphorus	10^{-11}
Electrolytic conductivity	Halogen compounds	10^{-10}
Ultraviolet	Aromatics	10^{-9}
Photoionization	Partially enhanced response to certain organic molecules as compared to flame ionization (not truly selective)	10^{-11} - 10^{-12}

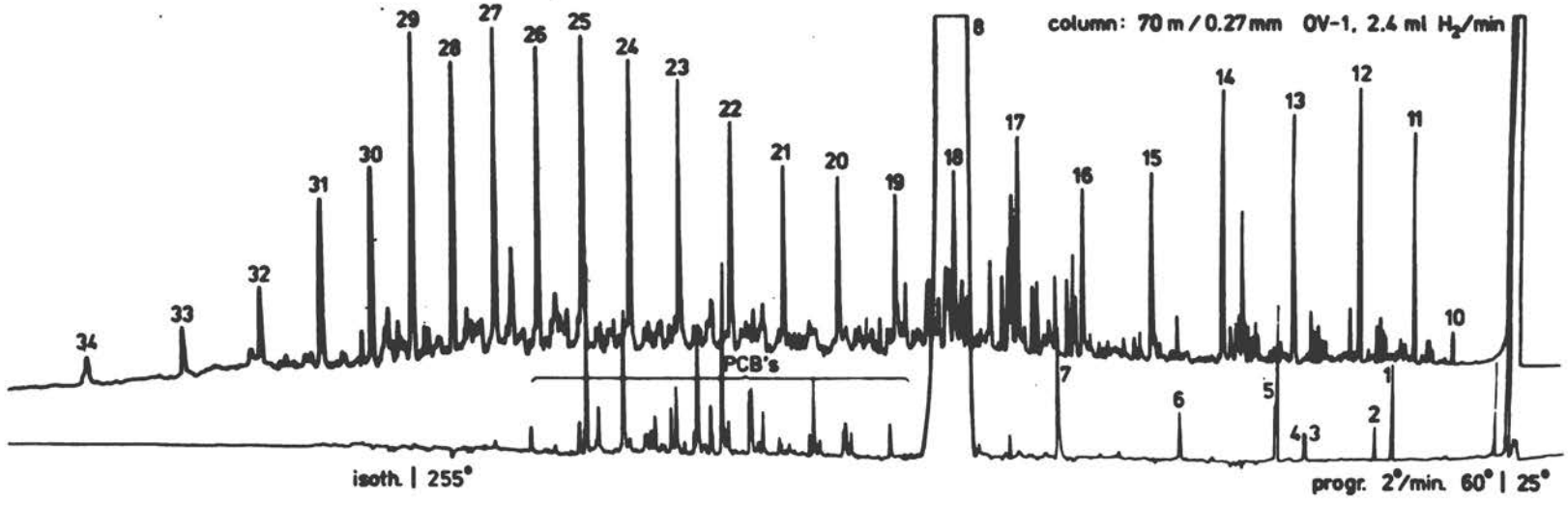

FIGURE 4. Chromatographic analysis of the nonpolar fraction of sewage extract. The organic components are simultaneously detected with the flame ionization detector (upper chart) and the electron capture detector (lower chart). PCB's = polychlorinated biphenyls. (From Grob, 1975. Reproduced with permission of Pergamon Press)

points: (a) even though mass-spectral techniques can give the ultimate in compound identification capabilities, screening with selective detectors may often provide initial information and supporting data; and (b) precisely measured chromatographic data help one to distinguish different isomers, which is difficult to do with mass spectrometry alone.

Once one has ascertained the identities of sample constituents, one must assess the health hazard for a given sample through reliable quantitation. The subject of measurement accuracy in complex sample matrices goes far beyond this presentation, but such accuracy is more often significantly impaired by the sample preparation steps than by the quantitative capabilities of modern gas-chromatographic instrumentation, which is accurate down to a few percent with calibrated mixtures. Only careful standardization of the overall analytical procedure can ascertain highly quantitative results.

LIQUID CHROMATOGRAPHIC ANALYSIS

Although nonvolatile compounds account for a large percentage of complex environmental samples, many major weaknesses exist in the related analytical chemistry. The rapid advances in high performance liquid chromatography (HPLC) have somewhat bridged the gap, but many general problems still persist, including (a) a lack of compound resolution comparable to gas chromatography; (b) the unavailability of universal detection means; and (c) a lack of on-line ancillary techniques

for compound identification. The frontier areas of modern analytical chemistry will be associated with development in these directions for some time.

HPLC techniques are highly valuable for certain environmental problems. Many industrial toxic substances are relatively nonvolatile (see, e.g., Hites and Lopez-Avila, 1979). Although acquiring HPLC separations at optimum levels is less straightforward due to mobile-phase variations, the method development for a limited number of known environmental pollutants can be a relatively easy task. On the other hand, screening efforts in this direction are likely to be more of a problem.

Resolving capabilities of HPLC have been recently improved through developments in microcolumn technology (for reviews, see Novotny, 1980; Scott, 1980). Efficiencies comparable to those routinely achieved in capillary gas chromatography are now available, but the analysis times in micro-HPLC are considerably longer. To overcome this problem, a significant reduction in the particle size for packed columns or the column radius for capillary HPLC columns must become feasible; both alternatives present a formidable technological challenge.

Furthermore, there is no universal sensitive detector for HPLC. In a number of practical analyses, this difficulty is overcome by the availability of certain selective detectors. Detection devices based on absorption spectrophotometry, spectrofluorimetry, and electrochemical phenomena are popular.

Some of these devices have detection sensitivities down to 10^{-12} grams, but such figures vary widely for different compounds. In a few cases, one can enhance detection sensitivity through a chemical alteration of solutes, and pre- and postcolumn derivatizations are becoming increasingly common.

However, identification problems with nonvolatile solutes separated by HPLC are particularly worrisome. Clearly, HPLC cannot match the availability of ancillary techniques for gas chromatography. Although possible utilization of liquid chromatography/mass spectrometry was vaguely mentioned during discussion of the Analytical Master Scheme (Donaldson and Garrison, 1979), it is not likely that such a combination will be fully developed for some time (Arpino and Guiochon, 1979). Development of various spectroscopic and electrochemical techniques for compound identification in HPLC is one of the most important tasks of modern analytical chemistry.

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The Characterization of Complex Mixtures Including Natural and Xenobiotic Organic Substances

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Many of the problems still faced in the daily pursuit of research aimed at elucidation of the molecular nature and function or dysfunction of living systems and their interactions with our biosphere concern the analysis of selected components in complex mixtures of organic substances or the identification of all of the components of the complex mixtures themselves. More often than not the components of interest are present only in trace levels, placing particular constraints on suitable methodology.

All biological systems utilize, modify, and excrete complex mixtures, which comprise a natural milieu. Some components originating from these natural sources can produce toxic effects in other species within the biological community (Kingsbury, 1980; Oehme et al., 1980). Superimposed on such a natural molecular environment (Eglinton et al., 1979) is an evergrowing assortment of anthropogenically derived complex mixtures (Horman, 1979; Risebrough, 1982; Safe, 1979). Some components are of concern because of their threat to the general ecological well being of our biosphere (Bowes, 1981); others may have the potential of continuing risk to human health (Burlingame et al., 1980; Horman, 1981; R.W. Miller, 1981;

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S.A. Miller, 1981; Richmond, 1981). In some cases, such components are known; in others, they are not (Risebrough, 1982).

Many general problems are encountered in studies of environmental chemistry of complex mixtures: they involve sampling and the matrix, contamination control, interlaboratory comparisons of complex mixture analyses, and correlations of known or unknown components from sample to sample and from environment to environment. In the context of potential risk to human health, there is then the determination of the extent of human exposure and its correlation with observed symptomatology. So, is the hard work really concentrated on devising the strategy for isolation, separation, and identification of complex mixtures? Is it in making an unambiguous link between one specific substance and the observed toxicity or between some small groups of substances and the observed toxicity? Or is the hard work ahead really empirical determination of the structure-toxicity relationships mediated by each species' metabolic individuality (the analog of structure-activity relationships in a pharmacological context)?

These are but a few of the difficult questions that only time and further knowledge can bring into better focus. At present, we know that particular physicochemical instrumental techniques have been the most successful and show the most promise among our analytical tools for providing clear answers, at least in (1) the chemical identification of the components of such complex mixtures, and (2) the metabolic activation of xenobiotic covalent lesion in cellular constituents. Since we are generally concerned with limited sample size and high analytical sensitivity, as well as concomitant molecular structure identification specificity, mass spectrometry in some form or other has become preeminent in this role in

concert with gas and liquid chromatography (Burlingame et al., 1980). This established position will continue and even grow in scope in the foreseeable future due to rapid technical advances in mass spectrometry.

However, a couple of important aspects concerned with identifying complex mixtures and elucidating the molecular mechanisms of toxicity and carcinogenesis pose problems for mass spectrometry alone and require the use of additional and supporting techniques such as chromatography and Fourier transform nuclear magnetic resonance spectroscopy. One of these aspects is the ability to distinguish isomers or congeners, such as for polychlorodioxins and the polyhalogenated biphenyls where the toxicity and potency of the mixture depend in dramatic ways on each congener's halogen substitution pattern on the aromatic rings as well as their relative quantities (Aust et al., in press; Goldstein, 1979). The substitution patterns are best determined by nuclear magnetic resonance or highly reproducible chromatographic retention indices obtained from standards, where sufficient chromatographic resolution permits the necessary high precision retention index measurement. The determination of precise stereochemistry is also a matter for nuclear magnetic resonance, helped sometimes by structural analogs and retention index information, as, for example, with the ultimate carcinogen associated with the hydrocarbon, benzo(a)pyrene (Yang et al., 1976).

Another significant aspect is the potentially toxic substance or mixture, which varies from species to species. Often this process produces an extensive mixture of metabolites (Gelboin et al., 1977), some of which are relatively stable electrophiles with the nasty habit of covalently binding to cellular nucleophiles (Miller and Miller, 1977). The elucidation

of their detailed chemical reactivity and mechanisms provides insight to permit understanding of the molecular basis of the nature of the toxicity observed, expressed primarily as covalent binding to a variety of cellular constituents, including proteins and the genetic encyclopedia, deoxyribonucleic acid (DNA). Therefore, from the points of view of both environmental exposure and intermediary metabolism, we face complex mixtures, some components of which covalently interact with cell machinery and express the toxicity syndrome with which we are eventually concerned.

Mass spectrometry has had a long and successful history in the study of complex mixtures. To my knowledge, the first work on mass spectrometric mixture analysis was done in 1940 by Hoover and Washburn (1940, 1941; Washburn et al., 1943). Using 1 hour of instrument time, they were able to analyze a nine-component mixture of C-5 and C-6 hydrocarbons in just 4 hours. The then-existing standard methods of separation using a fractionating column and a refractive index detector method would have taken 210 hours for the same mixture. The subsequent long, extensive record of the development of mass spectrometry for petroleum refining mixture analysis (Hamming and Foster, 1972) culminates in the utilization of low voltage electron impact techniques with high mass resolution to deal with the considerable heteroatom content of the various components of different crude oils (Lumpkin and Aczel, 1978). The invention of gas chromatography by James and Martin (1951, 1952) was essentially coincident with the recognition by Stevenson and Wagner (1951), Ryhage and Stenhagen (1963), McLafferty (1957), and Biemann (1962) (among others) that a mass spectrum consisted of an ensemble of gas-phase carbonium ion/radical chemical species characteristic of the original structure of the neutral molecule introduced

into the mass spectrometer. Development of this inherent potential for mass spectrometry as a structural tool for very small sample sizes thereupon exploded into the tremendously large analytical and research effort that exists today (Burlingame et al., 1980, in press; Mellon, 1981; Waller and Dermer, 1980).

The first coupling of gas-liquid chromatography to mass spectrometry was attempted in 1957 (Holmes and Morrell, 1957). The coupling of scientific computers to help chemists deal with the tremendous load of data on the nature of complex mixtures began to be used in the mid-1960s (Chapman, 1978), and other more chemically mild ("soft") methods of creating gas-phase molecular ions began and still continue to develop at a vigorous pace (Morris, 1981). These include the use of ion-molecule reactions ("chemical ionization"), of high field gradients to effect electron tunneling ("field ionization"), thermalized electron plasma ("negative chemical ionization" or electron attachment to electronegative species), and primary photon (laser) atom or ion bombardment. Also playing important roles are accurate mass measurement, for direct determination of the elemental composition of molecules and mixtures, and higher mass resolution and their usage in complex mixture analysis (Kimble, 1978; Meili et al., 1979). In the mid-1970s, the development of high pressure liquid chromatography was initiated and various attempts were made to couple it with mass spectrometry (Mellon, 1981). The coupled techniques are not yet analytically mature.

For all the known and unknown natural and anthropogenic substances that we would want to identify and study including xenobiotics covalently bound to cellular constituents, there are three basic categories: mixtures that are volatile or volatizable through chemical derivatization procedures;

mixtures of thermally and/or chemically labile substances and salts, which we may not yet have learned how to derivatize without sample loss or decomposition; and substances that can be isolated but require degradative procedures in order to generate mixtures of substances that fall into the first two categories. These degradative procedures can, of course, be mild and highly selective, thereby avoiding the creation of artifacts. Examples are the very careful enzymic procedures for carcinogens or drugs covalently bound to native DNA (Straub and Burlingame, 1981). They can also be very crude and disrupt the structural integrity of the molecules being investigated, e.g., the pyrolysis of whole cells, kerogen, and plastics (Jones and Cramer, 1977).

Techniques of fused silica capillary gas chromatography coupled with both nominal (Burlingame et al., 1980, 1982; Mellon, 1981) and high resolution mass spectrometry (Meili et al., 1979) for complete identification of complex mixtures in the first category have been developed to a high level of routine performance. Present advances in field desorption and fast neutral atom or ion bombardment techniques with double focusing mass spectrometers have made studies of free substances in the second category eminently feasible and tractable while concurrently extending the molecular size into the 3,000 to 5,000 dalton range (Burlingame et al., 1982). The third category must still be addressed experimentally by some degradative approach amenable to separation and identification by techniques used in the first and second categories. Clearly the eventual understanding of the molecular nature of toxicity in its most general sense will require dealing with samples and mixtures in all three contexts.

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Standardization, Validation, and Quality Control

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The introduction of sophisticated chemical instruments into the analytical laboratory in recent years has considerably enhanced our knowledge of the distribution of chemical pollutants in the environment. Techniques such as gas chromatography/mass spectrometry now enable us to identify extremely toxic compounds at picograms per kilogram (parts per trillion) levels. However, for these measurements to have any validity, analysts must adopt rigorous protocols to establish the qualitative and quantitative limits of the analytical method being used.

From the qualitative viewpoint, there are a number of examples in the literature where investigators have misidentified chemical pollutants. In 1957, severe mortality occurred among chickens from certain areas in the South and Midwest of the United States. Scientists eventually traced the source of the disease to a fat supplement in the chickens' diets. An intensive research program over several years resulted in the isolation of the toxic factor as a crystalline material. Using a combination of ultraviolet and infrared spectroscopy together with low-resolution mass spectrometry,

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the investigators initially identified the crystalline material as a partially saturated chlorinated phenanthrene of molecular formula, $C_{14}H_{10}Cl_6$ (Wooten and Courchene, 1964). In fact, x-ray spectroscopy later identified the compound as 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (Contrell et al., 1966). Proposal of the incorrect structure was a result of a failure to recognize the aromatic ether absorption bond in the infrared spectrum. This conclusion that oxygen was not present in the molecule led to additional misinterpretation of the mass spectral data. Scientists attributed fragement ions resulting from the loss of 63 and 126 mass units to the loss of C_2H_4Cl and $2(-C_2H_4Cl)$, respectively. In fact these fragment ions are due to the loss of -COCl and 2(-COCl), respectively. In retrospect, the analysts could probably have deduced the correct molecular formula by using high-resolution mass spectrometry, a technique which was then in its infancy but which has now proved to be one of the most powerful tools for the analysis of complex chemical mixtures.

In routine analyses for known compounds, problems of accurate quantitation are often more severe than those concerning qualitative identification. This is particularly true in trace organic analysis, where investigators use multistep cleanup procedures to isolate the compound (or compounds) in question. In one interlaboratory study, 10 to 21 laboratories analyzed oyster tissue homogenates for three

organochlorine pesticides [σ-benzene hexachloride (σ-BHC), γ-benzene hexachloride (γ-BHC), and dieldrin] (Hertz et al., 1978). Relative standard deviations varied from 200% for σ-BHC to 87% for dieldrin. Obviously data of this nature are not useful. This paper, outlines what I consider some of the major factors that could lead to an improvement in environmental analyses.

THE ANALYTICAL PROCEDURE

Sample

The analyst very often has no direct involvement in the sampling program. This is unfortunate since the collection, storage, and handling of samples are obviously starting points for improvement in the reliability of environmental analyses. In cases that involve multichemical analyses, these considerations may be of even greater importance because of variations in physical and chemical properties. I will not discuss here appropriate sampling methods in detail because they are the subject of another paper in this conference.

Analytical Personnel

Although it seems self-evident, the qualifications of the analyst are of paramount importance in determining the success or failure of an analytical procedure. This is true even for so-called routine analyses. If they are conducted in a "cookbook" fashion, the analyst may fail to pay attention to important details, such as ensuring that the sample is not lost

in solvent evaporation steps. Although the analyst may not have a complete understanding of statistics, he or she should have an elementary knowledge sufficient to carry on an intelligent dialogue with a trained statistician.

Methods

Selection of the appropriate method depends on both the nature of the sample matrix and the purpose for which the analytical result is intended. In terms of matrix, a method of analysis for an organic pollutant in biological tissues may be quite inappropriate for separating and analyzing the same pollutant if it is present on flyash. Concerning the end use of the data, if the purpose is to screen a large number of samples where there is no background information on the pollutant(s), a simple, rapid procedure such as radioimmunoassay may suffice. On the other hand, when a detailed knowledge of pollutant levels is required for health assessment decisions, the analyst may need to use a relatively sophisticated method that is not subject to interference from other compounds.

Quantitation

The instrument response (S) is related to the chemical compound concentration (C) by the equation, $S = g(C)$, where the response factor (g) is determined by instrumental calibration with standard compounds. Traditionally, the chemical compound's concentration is corrected for recovery losses that

occur during sample preparation. Under these circumstances reliable results can be obtained only when the recovery is high (>50%) and consistent. However, with the widespread use of ion-monitoring mass spectrometry, investigators can add compounds labeled with stable isotopes prior to cleanup. Both the unlabeled compound and its isotopically labeled analog will then incur similar losses through the procedure, so that the analyst can determine the unlabeled compound concentration by a simple ratio calculation:

$$C = x \div y\ (RC_i),$$

where C is the unlabeled compound concentration, C_i is the concentration of isotope-labeled internal standard, x is the peak height for unlabeled compound ion, y is the peak height for internal standard, and R is the relative response of internal standard and unlabeled compound. In cases where the isotope-labeled internal standard gives an instrumental response for the unlabeled compound and vice versa, the equation is modified to read:

$$C = [(x - R_a y) \div (y - R_b x)]\ RC_i,$$

where R_a is the relative abundance of unlabeled ion to labeled ion in the internal standard, and R_b is the relative abundance of labeled ion to unlabeled ion in the unlabeled compound.

The isotope-ratio method can in fact be used to calculate the chemical compound levels reliably when recoveries are

extremely low, as evidenced by recent data obtained in our laboratory. We fortified fireplace soot at two different levels with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF), together with [U-^{13}C]-TCDD[1] as an internal standard (Smith et al., 1981). We then determined recoveries after isomer-specific cleanup procedures relative to external standards of 2,3,7,8-TCDD and 2,3,7,8-TCDF, and we calculated the absolute level of 2,3,7,8-TCDD from the internal standard ratio method (see Table 1). Although the recovery levels, based on external standards, were extremely low due to the complex cleanup procedure, the ratio results for 2,3,7,8-TCDD were within 10% of the fortification levels.

We conducted similar analyses on replicate samples of carbonaceous material formed during the course of a transformer explosion (see Table 2). There was only a 4% variaton between the levels of 2,3,7,8-TCDD calculated for each replicate; however, calculations for 2,3,7,8-TCDF, which were based on the use of external standards and a 13% recovery factor, showed considerable variation.

APPLICATION OF QUALITY CONTROL TECHNIQUES

The quantitative error in any analytical determination is a composite of the precision or random error and the systematic

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[1]TCDD uniformly labeled with ^{13}C atoms.

TABLE 1

Recovery of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-Tetrachlorodibenzofuran (TCDF) from Fireplace Soot[a]

Sample No.	Fortification Level (µg/kg)		Recovery %		TCDD Ratio Calculation (µg/kg)
	TCDD	TCDF	TCDD	TCDF	
1	8	15	5.6	18	9.0
2	0.08	0.15	4.0	5.5	0.09

[a]Data from Smith et al., 1981.

TABLE 2

Levels of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-Tetrachlorodibenzofuran (TCDF) in Binghamton Transformer Soot[a]

Sample	Analytical Result (μg/kg) TCDD	TCDF	^{13}C-TCDD Recovery (%)
Environmental blank[b]	ND(<0.012)	ND(<0.085)	14
Binghamton soot #1	2.80	273	27
Fireplace soot	0.003	0.007	[c]
Binghamton soot #2	2.90	124	16

[a]Data from Smith et al., 1981.

[b]Based on a theoretical sample weight of 3.5 mg.

[c]Not calculated because there was an error in sample trapping during the high-performance liquid chromatography step.

or bias error. The random error results from day-to-day variations in uncontrollable variables, such as laboratory temperature, and is inherent in any measurement process. Since this error can be equally positive or negative, analysts can adequately describe it by statistical procedures applicable to a Gaussian distribution.

The systematic error is of considerably greater importance to the chemist, as it introduces an error which is fixed in direction. The objectives of a quality control program are to define the precision and bias of an analytical method and to ensure that the estalished error limits are not exceeded during the analysis of environmental samples (American Chemical Society, 1980; Youden and Steiner, 1975).

Standards

The availability of analytical standards is a prerequisite to developing valid methods of analysis. Investigators must establish the purity of the compounds before using them. The nature of the data required for this purpose will vary with the properties of the compound. For organic compounds a full scan mass, ultraviolet or infrared spectrum should be a minimum requirement. Although this information is generally available either from the supplier or from experiments conducted in the analytical laboratory, publications or reports describing analytical methods frequently omit it.

In addition to using isotope-labeled compounds for internal standards, investigators often add them in substantial

quantities to serve as carriers for the sample through the cleanup process. Under these circumstances, the isotopic purity of the internal standard becomes a limiting factor in trace-level analysis. If the labeled atoms are not of high enough isotopic purity, significant amounts of unlabeled compound may contaminate the standard.

Standard reference materials, containing defined levels of chemical compounds in a natural matrix, can also play a very important role in the development of reliable analytical methods. The National Bureau of Standards (NBS) has been involved for a number of years in providing these materials for inorganic compounds. A recent review article by scientists from NBS (Hertz et al., 1978) pointed out that the greatest difficulty in extending them to organic compounds involves the lack of suitable methods for certification. However, in view of the present emphasis on trace organic analysis, standard reference materials for organic compounds should become available in the future.

Instrument Calibration

Calibration consists of measuring signals from the analysis of defined quantities of a standard compound. To determine the error associated with this measurement process, investigators should measure at least three different concentrations of the sample in triplicate with the concentrations covering the entire range of those expected. If the response is linear, the analysts can plot the data as a regression line. When we use

ion-monitoring mass spectrometry to analyze TCDD in our laboratory, we have invariably found that we can plot results from the analysis of standard solutions over a dynamic range of 10^3 as linear calibration curves that pass through or close to the origin. Under these circumstances, a single-point ratio calculation is sufficient information for determining a response factor. However, if the calibration curve has a significant intercept, bias errors will be present, unless we make the calculations of sample concentrations directly from the calibration curve (Cardone et al., 1980).

Although it is proabably unnecessary to prepare a separate calibration curve on a daily basis, we should analyze at least one standard every day. We can then plot the result as a quality control chart; any signficant deviation in the instrument response may then necessitate a reevaluation of the entire calibration curve.

Sample Categories

Scientists generally evaluate the reliability of the complete analytical procedure (sample collection, storage, extraction, cleanup, and instrument response) using the following sample categories: laboratory or procedural blanks, field blanks, and fortified field blanks. Three or more replications are necessary for the blank samples and for several of the fortification levels in order to obtain data on the precision of the method. Although we can address the effects of instrumental analysis separately by calibration with

standards, Albro (1979) has suggested that analytical chemists often do not adequately consider extraction and cleanup as distinct steps.

A frequently erroneous assumption when samples are "spiked" (fortified) is that the spiking compound equilibrates with or distributes in the sample matrix in the same manner as the endogenous compound. If this does not occur, analysts may report artificially high recoveries. In the case of biological samples, radiolabeled compounds can often be administered in vivo, provided either that the compound does not metabolize or, in the event metabolism does occur, that the metabolites are not present in significant quantities in the tissue. We can then determine extraction efficiency by comparing the radioactivity after complete tissue destruction with the radioactivity obtained from the extraction procedure under evaluation.

With regard to validation of the cleanup method, if possible, we should evaluate each step in the method separately for recovery, reproducibility, and removal of interferences. We can then consider omitting those steps where recovery losses are not accompanied by a significant reduction in interfering compounds or sample matrix. During the course of analysis of field samples, we should intersperse validation type samples among them as quality control checks. There is a wide spectrum of opinion as to what constitutes the appropriate number of quality control samples, but 10% of the number of field samples is probably a reasonable number.

Limit of Detection

The concept of a detection limit (K) is based on the need to determine the minimum instrumental response from a sample that can be reliably detected. A descriptive equation relates the gross sample signal (S_τ), the field blank signal (S_b), and the variability in the field blank (σ_b): $S - S_b \geq K\sigma_b$. For convenience in measuring the limit of detection of individual samples, σ_b is generally replaced by the peak-to-peak noise in the area of the signal ($\sigma = \sigma_n$). Various values of K have been proposed (Currie, 1968; Kaiser, 1970), but a committee of the American Chemical Society (1980) has suggested a value of K = 3, which seems acceptable since the error associated with making a false positive or false negative decision is only 7%. However, it is difficult to obtain quantitative accuracy near the limit of detection. To obtain more accurate results, an additional limit, the limit of quantitation, should be established with a value of K = 10.

Acceptable Total Error

The total error is a combination of the bias error and the precision errors. To make an objective evaluation of different analytical methods, McFarren et al. (1970) have recommended that investigators judge the total error at the 95% confidence level by using the following formula: total error = 100 (d + 2S) ÷ μ, where d is the absolute value of the mean error, S is the standard deviation, and μ is the correct value. They considered methods that had total errors of 25% or less as

"excellent," and methods with total errors between 25% and 50% as "acceptable." Eckschlager (1972) has pointed out that, when d is not statistically different from zero, the equation reduces to: total error = $100(2S) \div \mu$. Midgley (1977) has also suggested that 2S may include more than 95% of the results and that analysts should use either 1.7S or 1.8S, depending on the relative values of d and S. With these modifications, the total error formula could serve as a useful means of evaluating analytical methods.

Qualitative Confirmation

Investigators must adequately establish the identity of the sample based on the physical or chemical properties of the compound. For instance, in the case of halogenated compounds analyzed by mass spectroscopy or gas chromatography/mass spectroscopy, we can use isotope-ratio measurements to determine the number of halogen atoms. Additional analysis can improve confidence in any identification by using a method that differs in some significant aspect from the method used for quantitative measurement.

Interlaboratory Studies

After development and validation of a method within a given laboratory, general acceptance of the method depends upon comparison with other methods in an interlaboratory study. Two such studies of analytical methods for TCDD residues in biological tissues were conducted recently under the direction of U.S. federal agencies. In the first study, the

Environmental Protection Agency provided four laboratories with cleaned up extracts of beef fat, which had been fortified prior to cleanup with TCDD at levels ranging from 0 to 81 pg/kg together with a fixed quantity of [2,3,7,8-^{37}Cl]-TCDD[2] internal standard. All four laboratories conducted their analyses with mass spectrometers tuned to 10,000 resolution. Three used packed-column gas chromatography/mass spectrometry systems; the fourth introduced samples into the mass spectrometer by direct probe. Accuracy and precision were measured by regression analysis.

Only one laboratory was able to analyze TCDD with a high degree of precision and accuracy (see Figure 1) down to a fortification level of 9 pg/kg; the others obtained results lacking in both precision and accuracy, as illustrated in Figure 2. The only significant difference between the gas chromatography/mass spectrometry systems used by these laboratories was the fact that one (laboratory F) had a mass spectrometer with an ultimate resolution of 20,000, whereas the other (laboratory H) had one that could be tuned to a resolution of 150,000. Under these circumstances laboratory H could achieve a resolution of 10,000 with relatively wide slit widths and thereby carry out analyses at high sensitivity.

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[2] TCDD labeled with ^{37}Cl atoms at the 2,3,7, and 8 positions.

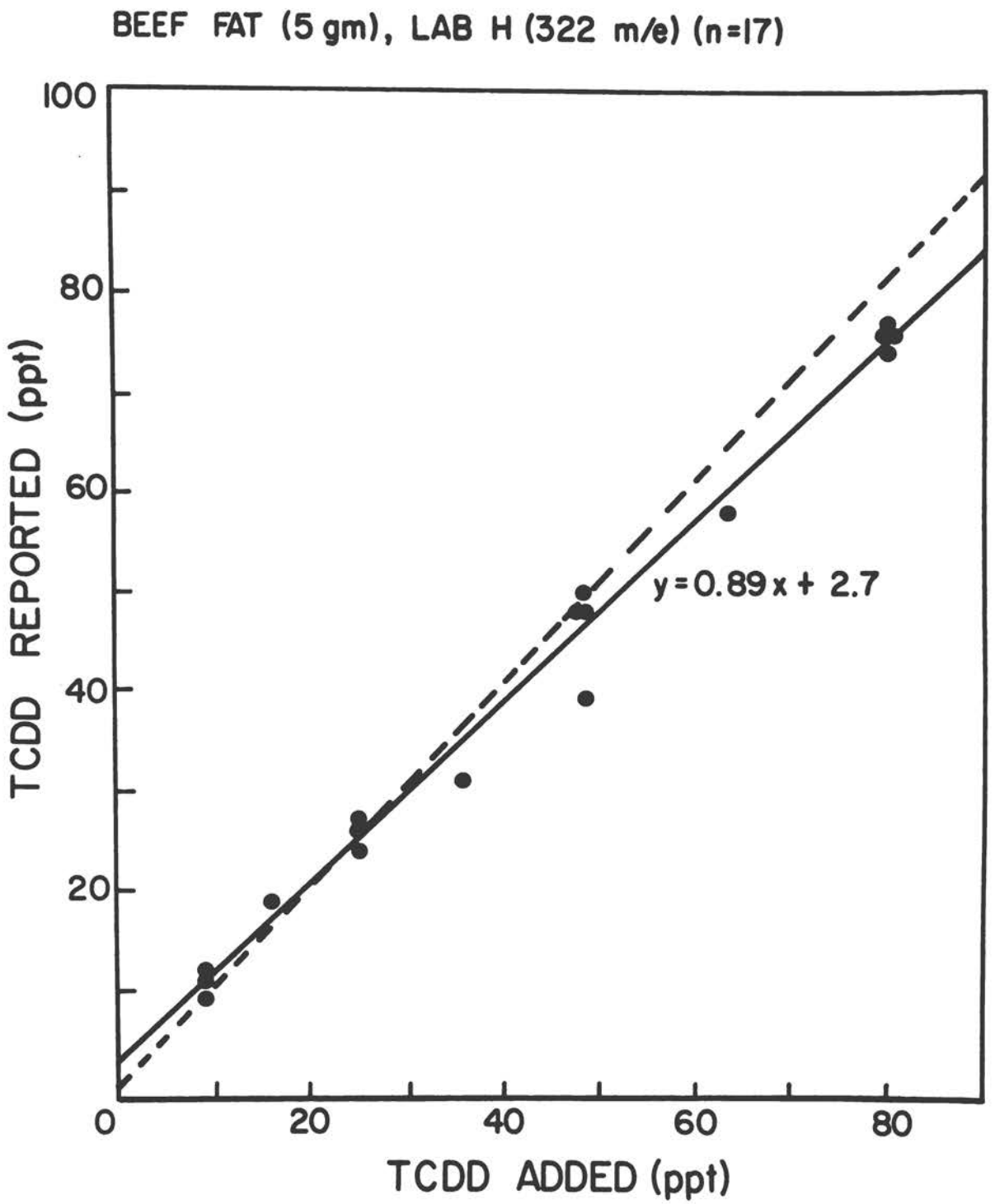

FIGURE 1. Results of gas chromatography/mass spectrometry analysis by "Lab H" of cleaned up extracts of beef fat, which had been fortified prior to cleanup with TCDD, plotted against theoretical result (broken line). (From Heath, 1979)

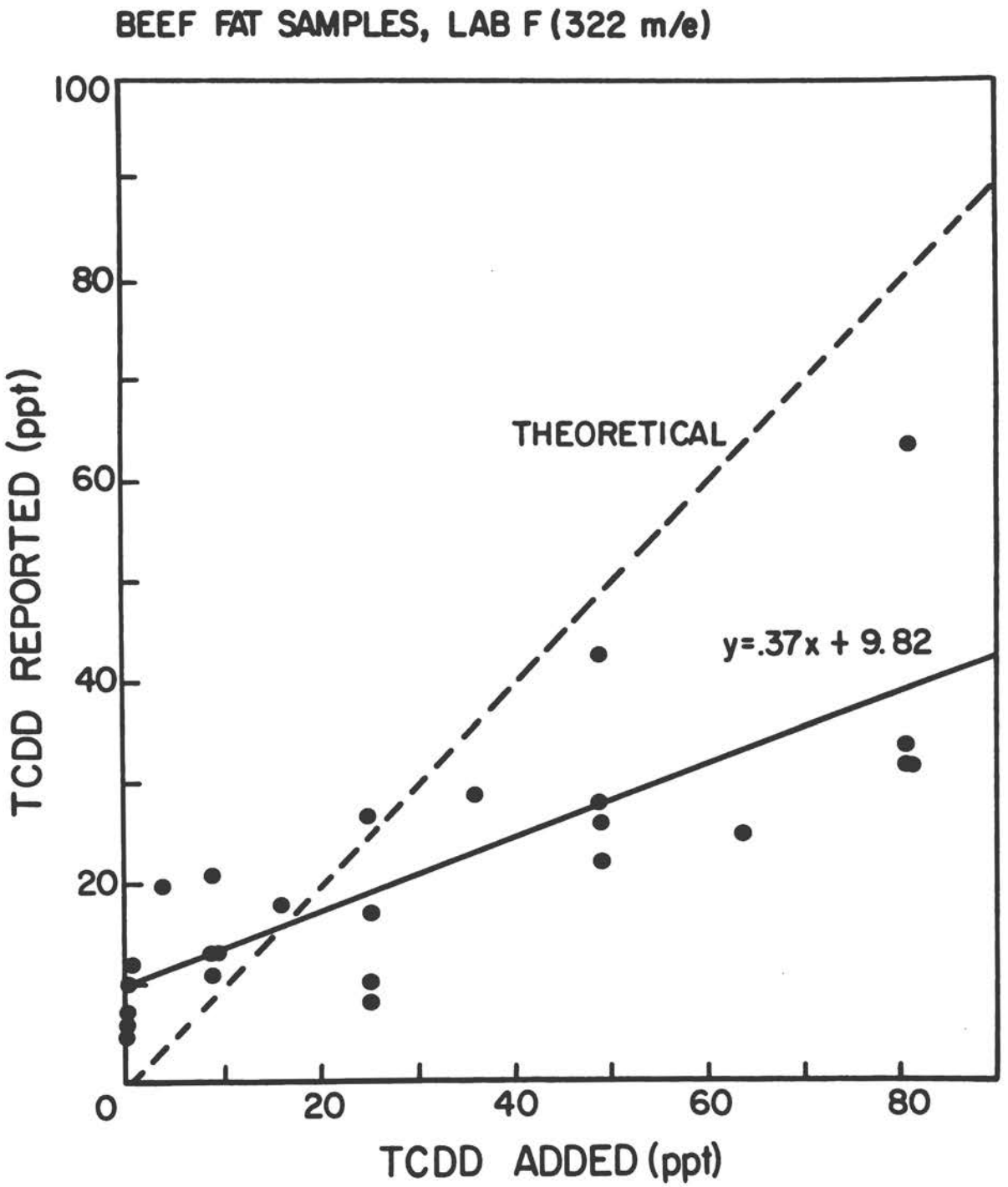

FIGURE 2. Results of gas chromatography/mass spectrometry analysis by "Lab F" of cleaned up extracts of beef fat, which had been fortified prior to cleanup with TCDD, plotted against theoretical result. (From Heath, 1979)

The second study focused on comparing the efficiency of various cleanup procedures, rather than evaluating the quantitative accuracy of instrumental methods of analysis (Brumby et al., 1981). Six laboratories received homogenates from fish collected in an area of potential TCDD contamination. Portions of the homogenates were fortified with TCDD, and the sample set consisted of the following:

Sample Number	Description of sample
1	sucker
2	sucker fortified with 105 (121) pg/kg TCDD
3	catfish
4	catfish fortified with 105 (121) pg/kg TCDD
5	catfish
6	catfish fortified with 105 (121) pg/kg TCDD

The cleaned up extracts were returned to the organizing laboratory for analysis by capillary gas chromatography/low resolution mass spectrometry. Twelve ions were monitored for confirmation of the presence of TCDD, and one of these ions, m/e 322, was used for quantitative measurements relative to the m/e 334 ion of the internal standard [$^{U-13}$C]-TCDD. Additional information on cleanup efficiency was provided by gas chromatography with an election-capture detector and full-scan gas chromatography/mass spectrometry.

Table 3 presents the ion-monitoring results. Three of the laboratories (C, F, G) provided cleaned up extracts in which TCDD could be quantitated and confirmed. Extracts from two

TABLE 3

Summary of Multiple-Ion-Detection Gas Chromatography/Mass Spectrometry Results of Study of Tetrachlorodibenzo-p-dioxin (TCDD) Extraction Cleanups at Seven Laboratories (A-G). (Confirmation of Identity; Quantitation in pg/kg)[a,b]

	A		B		C		D		E		F[f]		G	
Sample Number[c]	Conf.	Quant	Conf.	Quant.	Conf.	Quant.	Conf.	Quant.	Conf.	Quant.	Conf.	Quant.	Conf.	Quant.
1	No	5	No	6	No	-	No	-	No	-	No	-	No	9
2	No	67	No	89	Yes	77	No	-	No	-	Yes	67	Yes	47
3	No	34	No	42	Yes	57	No	-	No	-	Yes	25	Yes	22
4	No	188	No	99	Yes	128	d	d	No	-	Yes	113	Yes	117
5	e	e	No	53	Yes	38	d	d	d	d	Yes	45	Yes	56
6	No	178	No	199	Yes	107	d	d	d	d	Yes	100	Yes	96

[a]From Brumley et al., 1981.

[b]Confirmation of identity of TCDD occurs if the responses of the 12 monitored ions for the sample extract are consistent with the responses of the 12 monitored ions of TCDD standard. Quantitation is based on the observed responses at m/e 322 and m/e 334.

[c]See text for sample identity.

[d]Samples were not run due to large amounts of coextractives.

[e]Some or all of the sample was lost.

[f]Quantitation by external standard because of ^{13}C-TCDD carrier.

other laboratories (A, B) could be used for quantitative measurements with ions at m/e 322 and m/e 334, but there was no confirmation because interferences obscured many of the other monitored ions. The remaining laboratory used two different cleanup methods (D, E); in each case significant levels of coextractives inhibited quantitation or confirmation of the presence of TCDD.

Although the two interlaboratory studies had different objectives, together they serve to emphasize the critical relationship between the sample matrix, the cleanup, and the extraction method and the method of instrumental analysis. The most inefficient method in the fish study, Method D, was used to clean up the beef fat extracts in the first study. It is a relatively simple procedure involving base hydrolysis, acid partitioning, followed by two separate alumina chromatography steps. Clearly, laboratory H, using a very high-resolution mass spectrometer, was capable of accurately analyzing TCDD down to 10 pg/kg in beef fat extracts prepared by this method. On the other hand, laboratory E, which participated in both studies, had very erratic results when analyzing the same beef fat extracts. However, by using its own complex cleanup method, consisting of reagent-modified gravity-flow adsorption columns followed by two high-pressure liquid chromatography steps, laboratory F was able to produce extracts from fish that could be readily analyzed by gas chromatography/low resolution mass spectrometry.

CONCLUSIONS

Although environmental samples often consist of complex mixtures of chemicals, investigators can reliably detect trace quantities of individual chemicals by adhering to strict protocols for instrument calibration, method validation, and quality control. This paper has focused on one compound, 2,3,7,8-TCDD, in order to describe the considerable advantage inherent in the use of an isotope-labeled internal standard as a part of ion-monitoring mass spectrometry. However, if high recovery values can be obtained, then even in the absence of isotope-labeled internal standards, analysts can carry out multichemical analyses for closely related compounds with considerable accuracy and precision. For illustrations, see the work of Lamparski and Nestrick (1980) on the analysis of particulate matter for chlorinated dibenzo-p-dioxins.

Regulatory agencies are now attempting to develop analytical techniques for the study of a wide range of chemical compounds with different physical and chemical properties in industrial wastewaters and the like. In the interests of time and cost, these methods often involve minimal sample preparation and chromatographic separation, relying on the power of instruments such as mass spectrometers to differentiate between compounds. With traditional mass-spectrometric techniques, this may lead to inaccurate quantitation and even erroneous compound identification. However, recent developments in mass spectrometry, such as

metastable ion analysis, show considerable potential for compound identification in complex mixtures and may therefore play an important role in the future development of environmental analysis.

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Standardization, Validation, and Quality Control for the Analytical Determinations

S. Facchetti[1]

The determination of several pollutants found simultaneously is a complex problem, which has not been completely explored from the chemical point of view. Although in the literature there is no lack of information about the environmental level of various pollutants, often the wide range of values in the published measurements limit the value of the data. Where the adoption of similar methods allows comparative evaluation of the results, variations in environmental concentration may remain, which one can reasonably refer to the type of the source, to geography and climate, or to the urban or industrial nature of the area studied.

The reliability of sampling and analysis methods is also complicated by the diversity of the matrices to be analyzed and of the type of measurement imposed by multiple contamination. Therefore, criteria and procedures that guarantee the availability of reliable and comparable analytical data must be clearly defined, even if this is only one stage in the solution of problems stemming from contamination by multiple pollutants. This is particularly imperative for the definition of environmental quality criteria on which prevention directives can be based.

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STANDARDIZATION

The quantitative measurement of contaminants is difficult because of the large number of potentially interfering compounds. Therefore, the principal need is for well-tested methods and appropriate standards or reference materials.

Calibration involves checking physical measurements against accepted standards. Standardization consists of determining the response function $S = f(c)$, where S is the measured net signal that is a function of the given substance concentration (c). One should carry out regression analysis with at least five different concentrations of the calibration standards measured in triplicate, which must include the expected concentration of the substance in the field sample. The standardization must be done under the same conditions as the measurement process. If testing indicates a controlled condition, the corresponding restrictions must be included in the standardization process.

Measurement methods in routine use are often compromises in terms of cost, analysis time, and experience of the laboratory staff; thus, each selected method should be exhaustively pretested for sources of error, and controls specified if necessary. Then the methods should be retested during the measurement process by periodic analyses of the blanks, standards, and "spiked" samples to monitor the corrective conditions intended to prevent anomalous results. Multicomponent analysis systems that determine the abundance and distribution of the chemical elements and products present in samples, as well as the degree of natural variability, prevent useless concern for a

contamination level that may be natural to the system. The simultaneous analysis of many compounds reduces also the analytical error mainly related to the low number of sample preparations and offers the possibility of analyzing not only trace but also major compounds to present the results as ratios to one or more "stable" compounds.

Often several methods are used to measure the same sample, and the results can vary considerably from laboratory to laboratory. Therefore, definitive and reference methods are required. Many organizations are already involved in the standardization of methods, among others the American Society for Testing and Materials, the Association of Official Analytical Chemists, the International Organization for Standardization, Deutsche Industrie Norm, and Unificazione Industria Chimica. Reference methods are available, in principle, for most low molecular mass compounds. Individual laboratories must test the feasibility of such methods, however, to eliminate potential sources of systematic and random errors and to pay special attention to the matrix.

There must be support for the development of improved reference methods for the analysis of high molecular mass compounds. Interlaboratory comparisons frequently have shown a wide variation of results. This state of affairs would be considerably improved by the introduction of suitable reference materials, which should be produced from reference methods. The type of method would depend upon the material.

All reference materials are important, and any priority will of necessity be arbitrary. Nevertheless, a working order of priority would consider the importance of an accurate determination with particular reference to the toxicity of the products, the frequency of routine tests for which the specific reference material is applicable, and the general availability of instruments that do not provide absolute measurements.

In summary, the simplest procedure to obtain a true measurement probably is to calibrate the instruments by means of certified and appropriate reference materials. The nonavailability of appropriate reference materials has often given rise to poor interlaboratory comparability and lack of reliability. The large amount of expertise in this subject in several centers (see list on pp. 108-114 at the end of this paper) should facilitate the preparation of appropriate reference materials for the most important contaminants. However, because of possible differences between various manufacturers' preparations and between batches from the same manufacturer, a reference material characterized by a number of chemical and physical criteria would allow the manufacturers to adjust their test kits for quality control, thereby making them more reliable. Thus, there is a need for national reference materials and working standards linked to reference preparation.

The purity of a working standard should be documented, possible interferences determined, and the possible time-dependence of the composition of a standard measured to determine whether any change occurred during the analysis. There

are three categories of possible reference materials and working standards:

1. products in the pure state, alone or in a mixture, which can be used for instrument calibration;

2. pure products added in known quantities to different matrices (their use would allow checking of instrumental analysis, when this may be carried out directly on the matrix, and of extraction and purification procedures in conditions similar to those of the real sample); and

3. real samples, with certification of the presumed levels of contaminants by analyses carried out with different reliable methods and, when possible, averages of analytical results from several qualified laboratories. The three classes are linked with the extent to which they are used. The materials of the first group are particularly suitable for instrument calibration, those of the second group to the evaluation of interference from matrices, and those of the third group to checking the recovery of the contaminants linked to the constituents of matrices. These last are the most valuable because they allow a complete verification of the analytical method under real conditions. Even if their absolute levels are only assumed, they are still of service to the individual laboratory because they allow comparison and correction of their own performance with that of specialized laboratories equipped with more advanced instruments and techniques.

VALIDATION

Specificity

The use of suitable reference materials allows one to check the general capacity of the laboratory, especially to define the measurement error and to carry out the regular calibration of the instruments. The validity of the analysis of the individual sample should, however, be guaranteed by checks that have been properly designed and adapted to the particular determination. Protocols, including all analytical procedures, should therefore be available to obtain reliable results.

This is true for both sampling and analysis. For example, the sampling of airborne substances must account for the interrelationship between exposure and possible absorption by the exposed subject as well as the facility of execution, reproducibility, and significance of the sampling, which are always very important parameters.

The analysis must be as appropriate and specific as the sampling. The term specific, which might assume a different significance when one refers to inorganic or organic analysis, must be clarified with some examples. Suspended airborne particulates in general include aerosols with sizes between 0.01 and 50-100 μm. The range of the granulometry and the variability of the chemical composition, however, make an evaluation of the "total" sample ineffectual. Therefore, specific measurements are necessary for the breathable fraction and for the particularly soluble toxic chemical species. A correct analytical measurement of a given species in its soluble chemical form, determined in

its most easily assimilated granulometric fraction and not that excreted by the human body, is imperative. From this point of view the correlations obtained between pollutants such as lead and carbon monoxide or vanadium and sulfur dioxide, which have high correlation coefficients in well-defined urban situations, do not appear to be applicable. The correlation would allow us to move from the determination of the concentration of gaseous pollutants to that of corresponding heavy metals evaluated overall and not to the fraction adsorbed by the human body.

Such correlations are, however, useful for finding the origins of some pollutants. The same result is obtained with cluster analysis in metals. In fact, comparison of the different parameters shows that the closer the metals are, the more similar is their behavior and thus their origin. For example, trace aluminum, cadmium, copper, sodium, and potassium can be traced to foundries and iron, chromium, manganese, and cobalt to steel works.

Detecting the origin of the pollutant can also be accomplished by using labeled and isotopically differentiated compounds. This is a very specific technique, which is useful even for large-scale experiments.

Of course, specific analysis of constituents that are identified in their chemical and physical form is not always possible. The atmospheric particulate in the analysis of fibers is a valid example. Fibers can be elongated particles, as long as they are sufficiently small and have a length/diameter ratio greater than three. These include natural fibers, which are

minerals (asbestos, hydrosilicates, and silicates) or organics (vegetable products such as cotton, linen, jute, etc., and animal products such as wool, silk, etc.), and artificial fibers, which are inorganics (sulfates, carbonates, etc.) or organics (acrylics, polyvinyls, polyesters, etc.).

Identifying the types of fiber would provide valuable information in the study of pollutants in the industrial environment, but the difficulties and uncertainties of the analytical determinations are considerable, and often the results obtained with the various methods are not in agreement. Only the combined use of optical and instrumental methods[2] yields reliable, although approximate, evaluations valid for fibers of known composition. The differences between operators, the complexity of the calibration necessary, the interference of the presence of unknown compounds, and the very small quantity of sample normally available are all factors. Thus, distinguishing the fibers according to their form is insufficient for the identification of the various types, in particular, of those most noxious for man.

The problem of determining 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), which became of paramount importance in Italy after the 10 July 1976 accident in Seveso, provides a good

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[2]Optical methods involve use of microscopes, such as the polarizing microscope, chromatic dispersion microscope, phase contrast microscope, ultra-microscope, or reflected light microscope. Instrumental methods include x-ray diffraction, microprobe analysis, single crystal electron diffraction, analytical electronic microscopy, and infrared spectrometry.

example of specific organic determinations. 2,3,7,8-TCDD, one of the 22 possible isomers of TCDD, is the most toxic (Holmstedt, 1980), but there is no established minimum level for excluding the dangers of toxic effects, especially the carcinogenic and teratogenic effects on humans. Consequently, we should use determination methods that are specific and sensitive at a level of parts per trillion (ppt). However, the diversity of the matrices, the presence of interfering contaminants, and the necessity of separating 2,3,7,8-TCDD from the remaining 21 possible isomers make determinations of this isomer in environmental samples particularly difficult at the ppt level.

Many isomers of TCDD have gas chromatographic retention times close to that of the 2,3,7,8-TCDD isomer (Buser and Rappe, 1980). Therefore, gas chromatography can separate them only by the use of capillary columns covered with different liquid phases of such lengths (~50 m) that analysis times are long and routine application is awkward.

Thus, the determination of 2,3,7,8-TCDD at the ppt level in environmental samples requires instruments of high specificity and sensitivity. At present, there are three categories of analytical methods that can satisfy these requirements, based on the gas chromatography/mass spectrometry technique:

1. purification and chemical separation at a high degree of specificity through use of an analytical apparatus having a low resolving power;

2. purification and specific chemical separation through use of an analytical apparatus having a high resolving power; and

3. purification, specific chemical separation, and instrumental separation based on high performance liquid chromatography through use of a detecting apparatus having a high resolving power and high sensitivity.

The use of high resolution instruments distinguishes the second and third methods from the first. The use of analytical instruments having a low resolution is valid only in a preliminary screening phase (unless the sample had been subjected to an appropriately tested purification of very high specificity), which must be followed by checking the positive samples with a high resolution apparatus. The third method seems the most suitable for lowering the detection limit and probably is the only one capable, in routine conditions, of supplying specific measurements for the single TCDD isomer. In fact, one can separate the 22 isomers of TCDD by using high performance liquid chromatography in the combination reverse phase and normal phase; using it at the end of the specific purification cycle for the particular environmental matrix would improve the quality of the extract and thus yield a higher signal-to-noise ratio in the mass spectrometric measurement. There would be an advantage in using higher gains for the output signal of the spectrometer, which would lower the limit of sensitivity.

In summary, the following procedure should yield a sure quantitative measurement of the 2,3,7,8-TCDD present at a ppt level in environmental samples of any origin:

1. extraction, purification, and separation as per the

specific method for the particular matrix involved;

2. high performance liquid chromatography (HPLC) in reverse phase, with collection of the sample at the 2,3,7,8-TCDD elution time;

3. HPLC in normal phase, with collection of the sample at the 2,3,7,8-TCDD elution time.

4. gas chromatography with capillary columns;

5. simultaneous measurement with a high resolution mass spectrometer (resolution power $\sim$10,000) of the signal of masses 320 and 322 for native TCDD and of the mass corresponding to the most intense molecular ion of the labeled TCDD, if added (greater specificity would derive from the measurement of a third ion for the native TCDD--the molecular ion at mass-charge ratio 324 or the fragment ion at 257--and from the measurement of the most intense molecular ion of a second, differently labeled TCDD); and

6. signal-to-noise ratio greater than or equal to three.

The certainty of the identification of the 2,3,7,8-TCDD should be based on the following determinations:

1. retention time in gas chromatography equal to that of the standard 2,3,7,8-TCDD;

2. abundance of the isotopic molecular ions and fragments with values equal to those found for the standards;

3. intensity ratio of the signals for the molecular ions of the labeled TCDD of the sample equal to that expected from their concentrations or equal to that obtained by direct analysis of the initial solution of the labeled TCDD of the sample;

4. repetition of the entire procedure on at least 10% of the samples showing positive results; and

5. analysis of control samples free of 2,3,7,8-TCDD (blanks) treated in parallel with the samples examined.

Other Factors

In addition to the high specificity needed for analysis of the most toxic contaminants, other factors can invalidate the analysis. Before the invention of sufficiently sensitive methods, experts believed that people did not contain many contaminants in their bodies, except in cases of accidental poisoning. The development of sensitive analytical techniques should reveal the common occurrence of traces of contaminants in humans and their environment, particularly in food. However, reliable trace analyses depend primarily on accurately determined blanks and only secondarily on the accuracy of the method itself.

Often analyses of many contaminants are unreliable because of an unfamiliarity with the extent, sources, and control of contamination during sample collecting, handling, and analysis. Consequently, many published data contain gross positive errors, and the error noise in concentration data determined at trace levels obscures the meaning of most work. At trace levels, the analyst must know with certainty the magnitude of the contribution of the contaminants under investigation from each reagent, from air exposure, and from laboratory ware. Without appropriate precautions, sophisticated analytical instruments are ineffective. Thus, widespread use of clean laboratory practices

is imperative. All the sample pretreatment operations, which may include sieving, blending, crushing, drying, dissolution, dilution, filtration, and addition of preservatives, should be documented so that the treatment used can be duplicated. Procedures should use controls and calibrations to prevent random and systematic error and provide high recovery with minimum contamination, and the number of steps in the procedure should be kept to a minimum in order to reduce the possibility of errors.

Precision and accuracy of measurement give a clear indication of the quality of the analysis. Where measurements are conducted with working standards, excessive measurement variability indicates probable uncontrolled systematic errors. If precautions have been taken to eliminate the systematic errors, the remaining fluctuations are considered random and will determine the experimental precision. The absolute signal variability (σ) is defined by the standard deviation in the estimated net signal (Sx). This quantity should be based on at least 10 observations (Crummett et al., 1980).

The relative variability of analytical measurements increases as the substance concentration decreases. There are three regions of reliability--the levels of determination, detection, and uncertain detection--in descending order of reliability. The limit of detection is the lowest concentration of a substance that the analytical process can reliably detect. The observed signal (St) is the sum of the instrumental response (Sx) due to the presence of the substance (x) in the sample, plus

a response signal (Sb) due to the background contribution (e.g., St = Sx + Sb). The limit of detection should be located at least at 3 σ above the blank signal Sb (e.g., St $\geq$ Sb + 3 σ). A value of three is considered minimal, as it implies the risk of false positive decisions; a more conservative value (e.g., six) will decrease the risk of false results (Crummet et al., 1980). The measurements are unreliable when they produce an excessive number of false positives or false negatives.

Initial positive results on actual field samples can be evaluated by repeated analyses of subsamples from the same field samples. Agreement between replicate analyses above the limit of detection increases confidence in the measurement. However, final data are not validated until two or more independent methods provide consistent results.

The confirmation procedure should be highly selective and based on analytical principles for analytical conditions different from those used in the initial method. Thus, one gas chromatography-mass spectrometry method may be validated by another that differs in the chromatographic conditions, ionization technique, or detection system. The region for quantitation should be above the limit of detection. The recommended minimum value is 10_{σ}, e.g., St $\geq$ Sb + 10σ; signals less than 36 should be reported as not detected (Crummett et al., 1980).

The recovery rate of a method is usually derived from the measurement of spiked blanks containing known added

concentrations of the substance. Added to a blank sample, the substance may behave differently (typically showing higher recovery) from that in the field sample. Care should be taken when spiking the sample with an appropriate tracer. One cannot affirm that the recovery based on a sample spiked with a labeled compound has a certain percentage value until an assessment has been made of the conditions and solvents used for the addition of the tracer. One must facilitate the absorption of the internal standard by the material examined and endeavor to submit the tracer to extraction conditions that are as close as possible to those of the endogenous product.

Whenever possible, testing should include experiments on homogeneous working standards containing known amounts of a naturally incorporated substance. Unfortunately, the frequent lack of such samples is an important limitation in trace analysis. As the recovery rate falls, the measurement process becomes more dependent on the knowledge of the precision of the recovery at that concentration. It is preferable to obtain reproducible recoveries rather than high, but variable, ones. Great variations increase the likelihood of an external unforeseen cause, which may render the procedure uncontrollable. Low recovery methods may be satisfactory in the region of quantitation only if the accuracy and precision are established. Recoveries of less than 50% should be considered unreliable.

Other useful precautions can ensure results close to a true value. Because preserving biological or vegetable tissues in

unsealed containers in freezers may cause partial dehydration, the samples must be previously weighed; desiccation may prevent an exact correlation of the concentration of the compound with that of the original tissue. Care should be taken during the extraction phase, because different types of matrix could, at a trace level, produce drastically different results. Thus, methods tested for TCDD analysis for vegetable tissues having a high water content reveal definitely inferior and scarcely reproducible results in the case of cereal samples; the same problem exists when the type of soil or tissue varies. A thorough examination of the extraction phase for different samples is therefore important, as well as the selection of the volume and number of extractions and the most suitable apparatus for the extraction.

In summary, when decisions regarding the presence of contaminants are based on results of compositions near or below levels measured by conventional techniques, the analyses are subject to numerous difficulties including interferences. A strategy is needed to reduce the error. This includes minimizing the complexity of the procedure and evaluating the experimental variable, thus reducing the opportunities for error that may arise when the measurement process is sensitive to small changes in operation. The reliability of analytical information depends upon the rigorous fulfillment of all the requirements stated in a well-defined analytical protocol, including confirmation and validation of the measurements. If doubts arise, additional

analyses should be performed with other methods. Unusually high or low results should be validated by analysis of a duplicate subsample by the same method and a third subsample by a different method. Finally, accurate data are far more likely to be obtained when supported by the use of calibration and working standards. Field blanks and field samples should also be periodically analyzed.

QUALITY CONTROL

The term quality control usually refers to a procedure by which samples of known composition are periodically analyzed and the results statistically evaluated to determine the accuracy and precision, at least. Control samples, as similar as possible to the unknown material, should be randomly injected in the different series of analyses in order to measure them under the same conditions as the unknown material. Double blind samples, if not identifiable, could also be periodically analyzed.

Interlaboratory comparison of homogenized subsamples may indicate serious discrepancies due to undetected errors. Youden's correlation technique (Youden, 1960) could be used in order to distinguish between random errors and laboratory bias.

The basic objective of a quality control program is to ensure constant reliability of the results. Periodic checking and calibration of equipment should result in an exact knowledge of the precision and accuracy of the analyses and provide an incentive for additional improvements in the measurements. A good quality-control system offers the opportunity for improving

not only the analytical capability of the laboratory, but also the aggregate performance of its personnel. As it is difficult to teach laboratory personnel how to eliminate errors, a quality control system should also include control of the errors within the responsibility of the laboratory and procedures for recognizing variability. Thus, the quality control should extend from the collection of samples to the reporting of results. In this sense, the term quality control could be replaced by the terms quality assurance, proficiency testing, or performance evaluation.

Errors and variability in the analysis can be introduced at several stages. They could include the choice of the sample, method of collection, sample identification, storage containers, transport systems, subsampling, analytical procedure, poor specificity or inadequate sensitivity of the apparatus used, calculations, and reporting results. One should evaluate all these steps and propose adequate solutions for eliminating the largest errors.

Many minor aspects of the analytical system are often not considered, and the variability introduced at these stages is frequently underestimated. Some examples are contaminant-contaminant interactions, the laboratory conditions (i.e., noise levels, temperature, humidity, cleanliness, etc.), a constant high work load of the laboratory staff, lack of involvement of the senior staff members, and difficult relationships between various members of the staff.

The variability of a method is usually established under optimal conditions by the most skilled operator working with sufficient time in an ideal environment and using an appropriate apparatus for specific measurement, checked reagents, and homogeneous samples. Although the variation measured under such optimal conditions is important for evaluating a method, knowledge of the variation of a method determined in routine conditions by an average operator on an average day is also important. The quality control system therefore must provide accurate assessment of both routine and optimal condition variances.

Because most analytical errors occur within individual laboratories rather than between experienced laboratories, adequate analytical performances would best be achieved by paying increased attention to internal quality control and by making a periodic review of the procedures. An internal quality control should include provision of representative samples and controls, use of replicate samples, and correction of departures from standards of quality.

Difficulties in the external quality control could derive from the methods chosen, which may not be homogeneous. In principle, the method must be sufficiently sensitive, precise over the entire range of concentrations, and without interference from other compounds. In practice the choice of methods is often based on the availability of instruments, on their cost, and on the experience of the laboratory staff. Therefore, results

obtained by reliable methods are compared with others less reliable. To avoid this inconvenience the results obtained by reliable methods could be taken as reference values. Mass spectrometric methods are generally considered to be the most reliable and specific, if operated in high resolution and multidetection mode, but they require access to expensive equipment and skilled operators. The combination of internal and external quality controls is an effective means of improving and sustaining the quality of determinations. Therefore, regular quality control programs will eliminate at least one uncertainty in decision-making--the reliability of the results obtained.

CONCLUSIONS

Precise and accurate measurements depend on the availability of proven methods, proper equipment, and individual skill. No measurement program should lack a well-designed measurement process established among the analysts, the statisticians, and the scientists who will use the data.

Accuracy is supported by the use of reference materials and participation in interlaboratory comparison activities. Performance testing, based on the use of working standards, is needed to monitor the recovery and variability in measuring samples and blanks. A complete report should provide sufficient and pertinent information on the sample, analytical procedure, instrumental measurements, and data treatment as well as on the

possible interferences that can arise at any stage in the analytical process. Accurate chemical analysis cannot be based only on the performance of sophisticated and sensitive instruments. Thus, modern analytical chemistry requires detailed protocols on the measurement system, sensitive and specific methods, permanent validation process, and systematic use of quality control procedures, which ensure the validity of the overall analytical measurement process.

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Organizations Marketing Various Types of Reference Materials

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Milwaukee, Wis., U.S.A.

All Union Scientific Research
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PART II: ENVIRONMENTAL INTERACTIONS

Role of Chemical Interactions in the Assessment of Multichemical Contamination

Morton Lippmann and Paul J. Lioy[1]

Chemicals released into the air, surface waters, and soil will generally react with other chemicals in those media. The resulting products will frequently react with other chemicals, and complex series of reactions may continue along extended physical transport pathways before the materials find semipermanent storage sites in terrestrial soils or aquatic sediments. The pathways and transit times for some pollutant chemicals are relatively simple and reasonably well-understood. For example, carbon monoxide (CO) can only react with oxygen (O_2) to form carbon dioxide (CO_2) or be taken up and metabolized by the biosphere. Its atmospheric oxidation rates and residence times have been described (National Academy of Sciences, 1977a; U.S. Environmental Protection Agency, 1979).

Another gas-phase combustion product, sulfur dioxide (SO_2), has a much more complex series of atmospheric interactions. It undergoes oxidation to sulfur trioxide (SO_3), via reactions with free radicals such as the hydroxyl, the hydroperoxy, and the methoxy radicals (HO, HO_2, and CH_3O_2), by reaction with ozone (O_3) and alkenes, by

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reactions after dissolution into aqueous droplets, and by reactions on the surfaces of graphite and soot particles. Reactions within droplets and on surfaces depend greatly on their compositions. The sulfur trioxide formed in the gas phase reacts in milliseconds within water vapor to sulfuric acid (H_2SO_4), a low-vapor-pressure droplet aerosol that is considerably more toxic than sulfur dioxide. As an aerosol, sulfuric acid has a longer residence time within the atmosphere than does sulfur dioxide. Thus, the transformation, by whatever route, results in a greater potential for health effects in downwind populations. On the other hand, further chemical reactions take place, primarily neutralization of the acid with ammonia (NH_3) to produce ammonium sulfate [$(NH_4)_2SO_4$], which greatly reduces its potential health effects.

In this paper, we shall discuss further the pathways and reaction rates for this complex series of events within the atmosphere as prime examples of chemical interaction. Another example is the photochemical sequence of reactions that lead to the formation of ozone. In this case, the toxic endproduct is highly reactive in comparison to the primary precursor pollutants, i.e., NO_x [nitric oxide (NO) plus nitrogen dioxide (NO_2)] and hydrocarbons, and in comparison to its reaction products. In each of our two examples the most toxic species, i.e., sulfuric acid and ozone, can be persistent only when the atmosphere is enriched with their precursors and the

temperature and radiant conditions are right for their continued formation.

Chemical interactions affecting human exposure and environmental quality also occur in surface waters and soils. However, these situations are usually much more site-specific than the two examples selected for discussion, and there are few situations where there is sufficient information available for the development of a good case history. Thus, we will limit this presentation to atmospheric chemical interactions where there is a reasonably complete body of relevant data and which affect a very large number of people.

PHOTOCHEMICAL SMOG FORMATION

The formation of ozone and other photochemical smog products, including high concentrations of fine particles and a variety of eye irritants, is a complex process. It takes place through a series of chemical reactions and depends upon the presence of sunlight and the right mixture of precursor pollutants. Smog episodes are often observed on hot sunny days, and the concentrations of products usually increase as the air mass travels from dense urban centers to areas downwind. Most of our knowledge of ozone formation mechanisms comes from laboratory chamber research (Altshuller and Bufalini, 1971; Leighton, 1961; National Academy of Sciences, 1976). These investigations used various hydrocarbon-nitrogen oxide-air-sunlight mixtures, and, although the details of each experiment differed, they all produced temporal patterns of

pollutant concentrations similar to that shown in Figure 1.

In the first phase, there is the conversion of nitric oxide to nitrogen dioxide, and the second phase finally produces ozone. Mixing and ventilation can affect the buildup of pollutants within the atmosphere as well as the transport of pollutants and their reaction products to areas downwind of the precursor sources (Lioy and Samson, 1979; Vukovich, 1977). As summarized in the ozone/oxidant criteria document (U.S. Environmental Protection Agency, 1978), the net result of the ozone production scheme can be represented by the following main reactions:

$$NO_2 + h_\nu \rightarrow NO + O \quad (1)$$

$$O + O_2 + M \rightarrow O_3 + M \quad (2)$$

$$O_3 + NO \rightarrow NO_2 + O_2 \quad (3)$$

$$XO_2 + NO \rightarrow NO_2 + XO \quad (4)$$

$$2NO + O_3 \rightarrow 2NO_2 \quad (5)$$

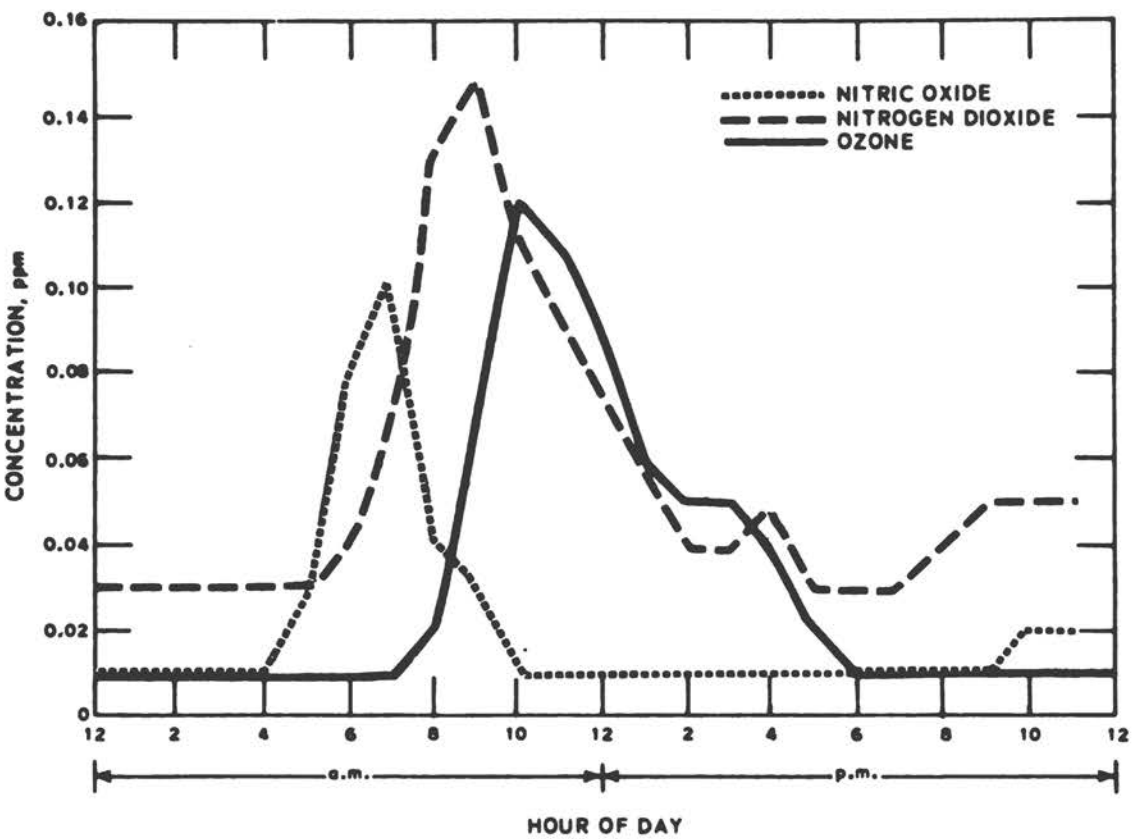


FIGURE 1. Diurnal variation of nitric oxide, nitrogen dioxide, and ozone concentrations in Los Angeles, 19 July 1965. (Reprinted from Air Quality Criteria for Ozone and Other Photochemical Oxidants, National Academy of Sciences, 1977)

where X is equivalent to hydrogen or a free radical.

There are many pathways within photochemical processes, and more research is needed to determine the products of individual organic or inorganic reactions and the concentrations of the free radical products. At present, many products cannot be determined directly; they may be a result of thermochemical considerations (National Academy of Sciences, 1977b).

From the steps illustrated in equations 1 through 3 and Figure 2, the nitric-oxide-scavenging reaction effectively precludes the buildup of concentrations. However, ozone does accumulate because other reactions are competing for the available nitric oxide molecules (Graedel, 1980). The most significant of these nitric oxide conversion reactions involve free radicals, which also will attack volatile organic carbon to produce more radicals and other partially oxidized products. The radical species are normally produced in a photochemically active atmosphere. For volatile organics, Figures 3a and 3b present an example of the major pathways for reaction by the trans-2-butene scheme.

A number of researchers have adopted a steady-state hypothesis for ozone accumulation using equations 1 through 3. The solution of the equations yields the following:

$$[O_3] = \frac{k_1 [NO_2]}{K_3 [NO]}$$

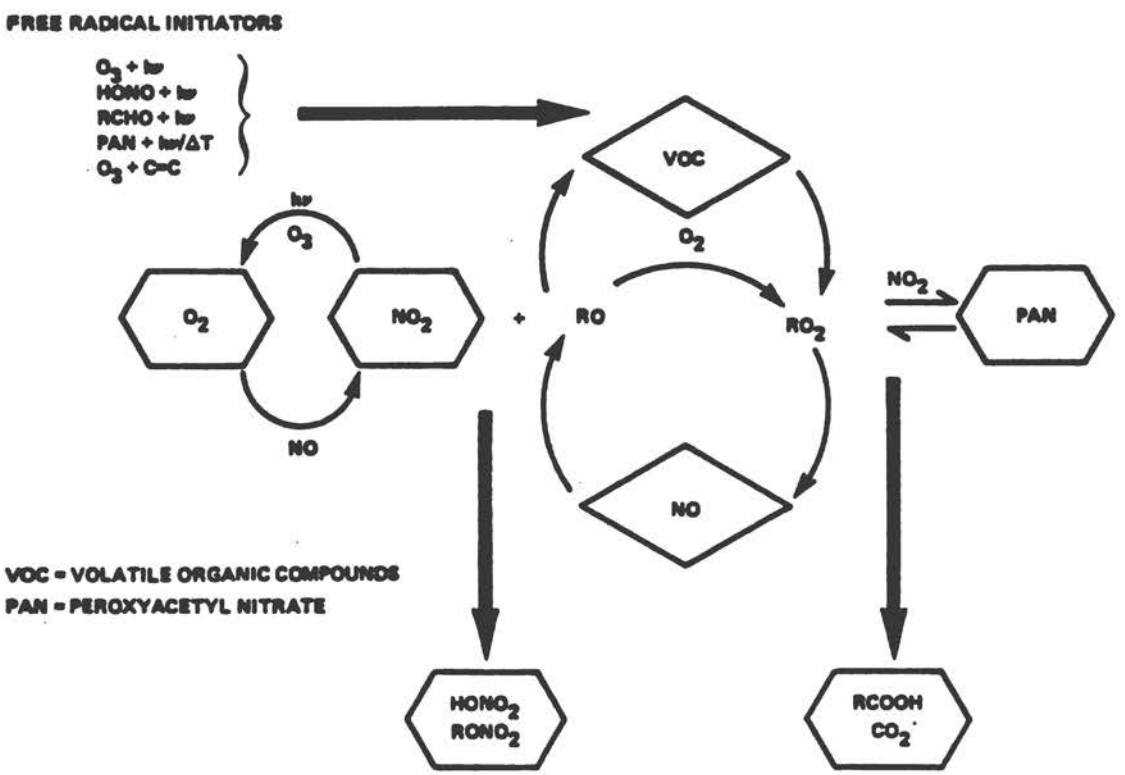


FIGURE 2. Schematic of the polluted atmospheric photo-oxidation cycle. See text for details. (From Air Quality Criteria for Particulate Matter and Sulfur Oxides, U.S. Environmental Protection Agency, 1981)

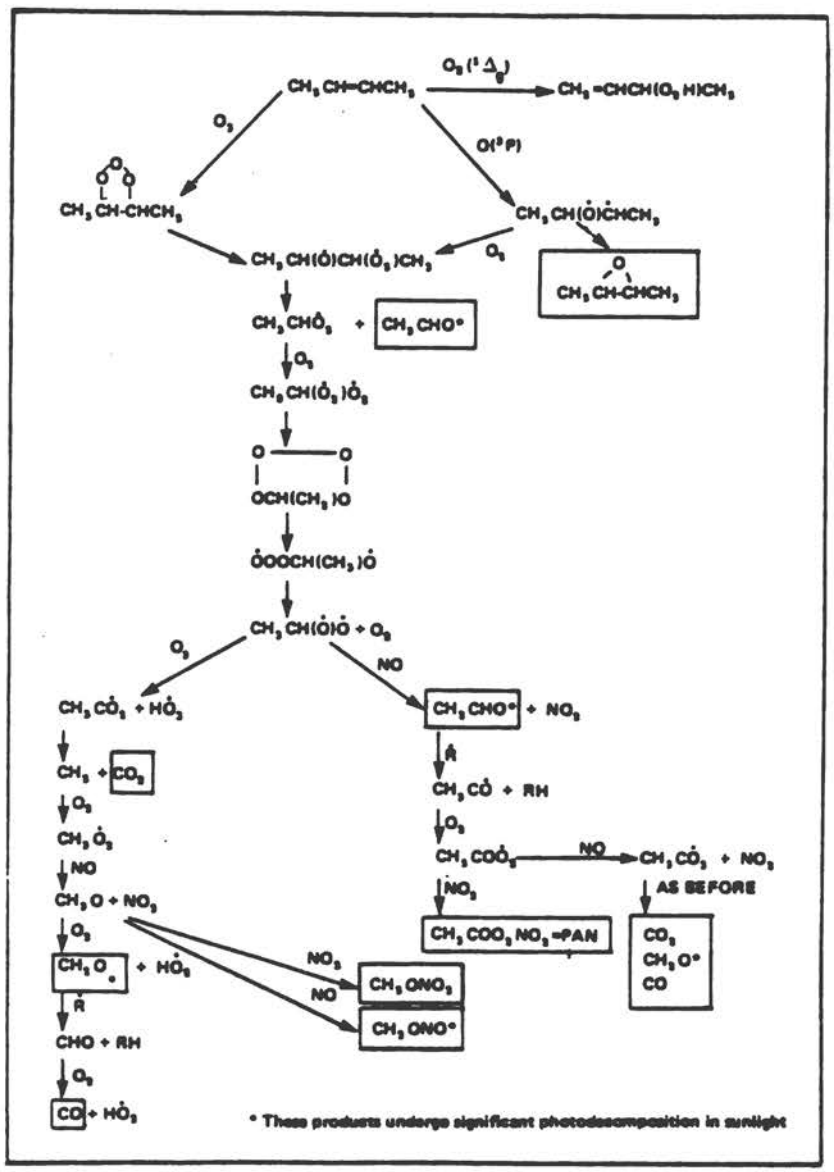


FIGURE 3a. The major reaction paths for the degradation of trans-2-butene in an irradiated NO_x-polluted atmosphere. (Reprinted from Air Quality Criteria for Ozone and Other Photochemical Oxidants, U.S. Environmental Protection Agency, 1978)

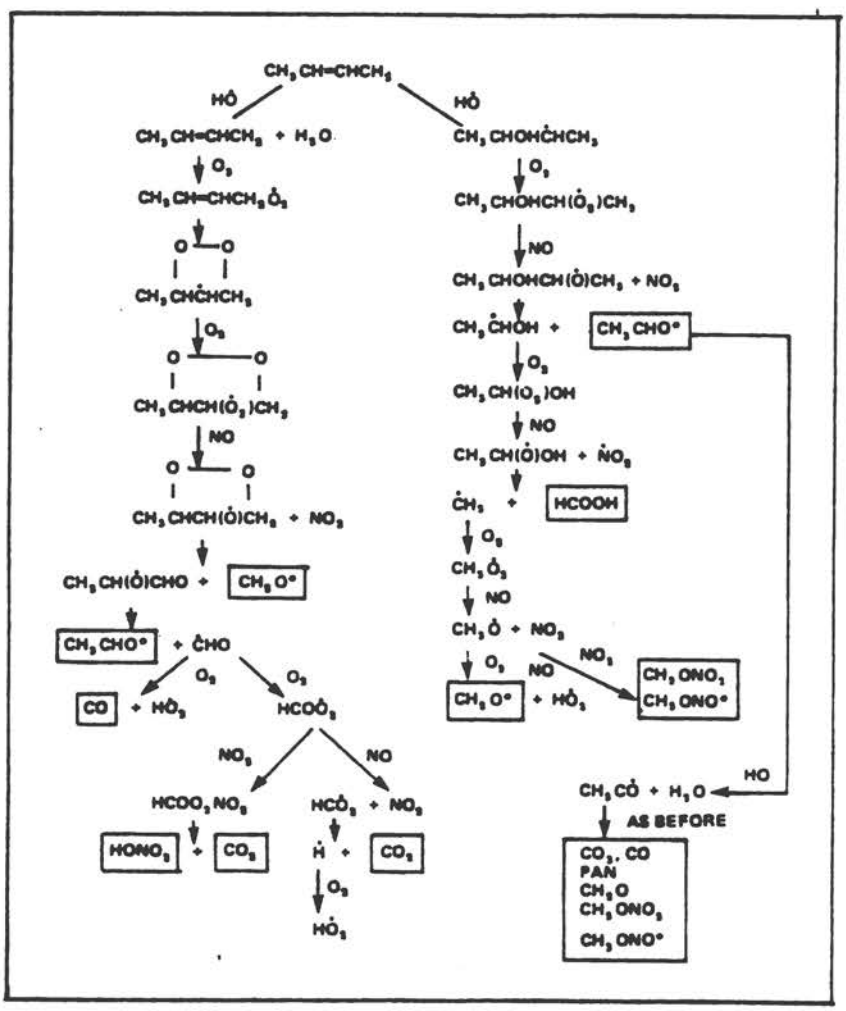


FIGURE 3b. Continuation of the major reaction paths for the degradation of trans-2-butene in an irradiated NO_x- polluted atmosphere. (Reprinted from Air Quality Criteria for Ozone and Other Photochemical Oxidants, U.S. Environmental Protection Agency, 1978)

where K_3 is the rate constant for reactions 2 and 3, and k_1 is the dissociation constant for nitrogen dioxide. Atmospheric studies have essentially verified this equation within the stochastic bounds of a turbulent atmosphere and illustrate the necessity for nitrogen dioxide buildup before ozone accumulation commences. This equation also predicts the ozone buildup curve in Figure 1, since a high ratio of nitrogen dioxide to nitric oxide is necessary for ozone concentrations to increase rapidly.

In terms of other chemical-chemical interactions, advances have been made in defining the identity, sources, and role of free radicals produced during the reactions of hydrocarbons and NO_x in the photochemical-smog formation mechanism. These radicals include the hydroxyl, hydroperoxy, and alkoperoxy radicals (RO_2), which are important (see equation 4) in the oxidation of nitric oxide to nitrogen dioxide (National Academy of Sciences, 1977b). However, the ozone reaction schemes used for photochemical smog models indicate that these radicals also participate in hydrocarbon and nitric oxide reactions that produce aldehydes, nitric acid, hydrogen peroxide, nitrous acid, peroxyactylnitrate (PAN), and more radicals (Dimitriades and Altshuller, 1977; Graedel, 1980; Winter et al., 1979). Because of the complex mixtures of hydrocarbons in the atmosphere, each reaction has a different rate, which will result in varying yields of these products. Some examples of the nitrate formation mechanisms are shown in Tables 1a and 1b;

TABLE 1a

Reactions Potentially Involved in Nitrate Formation

Species	dNO_2/dt, or Rate Constant (ppm/min)[a]
Nitrogen oxides	
1. $O_3 + NO \rightarrow NO_2 + O_2$	2.7×10^{-2}
2. $O + M + NO \rightarrow NO_2 + M$[b]	--
3. $RO_2 + NO \rightarrow NO_2 + RO$	2.5×10^{-3}
4. $O_3 + NO_2 \rightarrow NO_3 + O_2$	-4.0×10^{-4}
5. $NO_3 + NO_2 \rightarrow N_2O_5$	-1.0 to -23×10^{-4}
Volatile acids	
6. $N_2O_5 + H_2O \rightarrow 2HONO_2$	2×10^{-5}
7. $HO + NO_2 + M \rightarrow HONO_2 + M$[b]	-10^{-5}
8. $NO + NO_2 + H_2O \rightarrow 2HONO$	--
9. $HOSO_2O + NO \rightarrow HOSO_2\,ONO$ $+ H_2O \rightarrow H_2SO_4 + HONO$	--
10. $HOSO_2O + NO_2 \rightarrow HOSO_2\,ONO_2$ $+ H_2O \rightarrow H_2SO_4 + HONO_2$	--
Gaseous nitrates	
11. $NH_3 + HONO_2 \rightarrow NH_4NO_3$	$\sim 10^{-6}$
12. $RO_2 + (N_2O_5) + (NO_2) \rightarrow R^1C(=O)ONO_2$ $R^1C(=O)ONO+ \ldots$	-10^{-3}

[a]Typical for smog reactant concentrations in the first hour of reaction (e.g., Calvert and McQuigg, 1975).
[b]M = 1 atm N_2.

TABLE 1b

Aqueous Reactions of Nitrogen Oxides

13. $N_2O_5 + H_2O(\ell) \rightarrow 2H^+ + NO_3^-$
14. $NO + NO_2 + H_2O(\ell) \rightarrow H^+ + NO_2^-$
15. $2NO_2 + H_2O(\ell) \rightleftarrows H^+ + NO_3^- + HONO$
    $HONO + OH^- \rightarrow H_2O + NO_2^-$
16. $2NO_2^- + O_2(aq) \rightarrow 2NO_3^-$
17. $NO_2^- + O_3 \rightarrow (aq)NO_3^- + O_2$
18. $2NO_2 + H_2SO_4 \rightleftarrows HNOSO_4 + HNO_3$
    $HNOSO_4 + H_2O(\ell) \rightleftarrows HNO_2 + H_2SO_4$
    $3HNO_2 \rightleftarrows HNO_3 + 2NO + H_2O$
19. $RONO_2 + H_2O(\ell) \rightarrow H^+ + NO_2^- + R^1OH$

of the gas-phase reactions, numbers 6 and 7 are the most important in nitric-acid vapor formation.

Clearly, free radical species, especially the hydroxyl radical, play an important role in producing ozone and other potentially irritating gaseous and particulate species. Moreover, the supply of nitrogen oxides is a limiting factor in the entire photochemical-smog production mechanism since, in urban areas, the concentration of hydroxyl radical is dependent upon the reaction $NO + HO_2 \rightarrow NO_2 + HO$.

The details of the free radical reactions remain uncertain with respect to (a) the actual rate constants for all the reactions of hydroperoxy radical and alkoperoxy radicals; (b) the reaction sequence following hydroxyl-radical addition to olefins; and (c) the details of reactions involving alkyl and alkoxy radicals (National Academy of Sciences, 1977b). Table 2 lists compounds typically observed in photochemical smog, and Table 3 lists suspected compounds. These products will participate in the generation of more radicals and other stable products within various reaction chains. Furthermore, if other reactive chemicals, such as sulfur dioxide, are present, other reaction chains can also be initiated. Ultimately, relatively stable products such as sulfuric acid, nitric acid, and organic aerosols are produced and remain for some time within the atmosphere.

The organic aerosols include compounds containing aldehyde, carboxyl, and other functional groups (Hidy et al., 1980). Chamber studies have demonstrated olefinic reactions with ozone

TABLE 2

Compounds Observed in Photochemical Smog

Compound	Formula	Typical Conc.(ppm)	Maximal Conc.(ppm)	Reference
Ozone	O_3	0.1	0.6	U.S. Environmental Protection Agency, 1978
Peroxyactylnitrate	$CH_3COO_2NO_2$	0.005	0.2	U.S. Environemntal Protection Agency, 1978
Hydrogen peroxide	H_2O_2	--	0.18	Bufalini *et al.*, 1972
Formaldehyde	CH_2O	0.04	0.16	Altshuller and McPherson, 1963
Higher aldehydes	RCHO	--	0.36	Renzetti and Bryan, 1961
Acrolein	CH_2CHCHO	--	0.11	Renzetti and Bryan, 1961
Formic acid	HCOOH	--	0.05	Hanst *et al.*, 1974

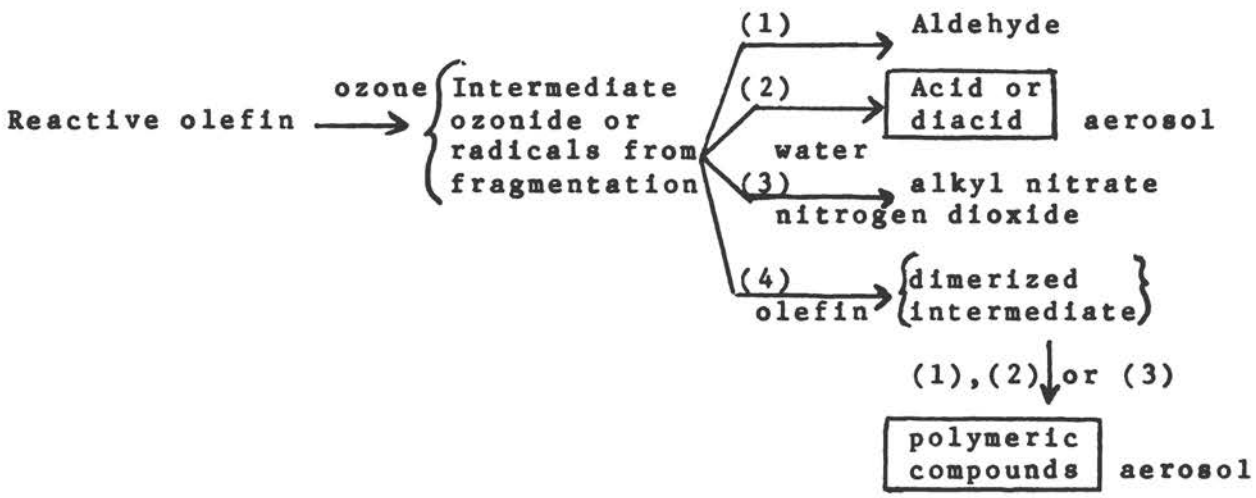
TABLE 3

Compounds That May Be Formed in Photochemical Smog

Compound	Formula	Possible Synthesis	Reference
Peroxybenzoylnitrate	$C_6H_5COO_2NO_2$	$\phi COO_2 + NO_2$	Heuss and Glasson, 1968
Nitric acid	$HONO_2$	$NO_2 + OH$	Hanst et al., 1974
		$N_2O_5 + H_2O$	Morris and Niki, 1973
Organic hydroperoxides	ROOH	$RO_2 + HO_2$	Demerjian et al., 1974
Organic peracids	$RCOO_2H$	$RCOO_2 + HO_2$	Demerjian et al., 1974
Organic peroxynitrates	RO_2NO_2	$RO_2 + NO_2 + M^a$	Demerjian et al., 1974
Ozonides	O_3-olefin	O_3 + olefin + M^a	Atkinson et al., 1973
Ketene	CH_2CO	O_3 + olefin	McAfee et al., 1974
Nitrous acid	HONO	OH + NO	Cox et al., 1976; Atkinson et al., 1975
Pernitric acid	HO_2NO_2	$NO_2 + HO_2 + M^a$	Levine et al., 1977; Niki et al., 1977; Graham et al., 1977
Pernitrous acid	HO_2NO	$NO + HO_2 + M^a$	Cox and Derwent, 1975
Organic nitrates	$RONO_2$	$RO + NO_2$	Darnall et al., 1976
		$RO_2 + NO$	Darnall et al.,1976

[a]M represents any molecule that takes part in the three-body process.

that produce low-vapor-pressure dicarboxylic and monocarboxylic acids and organic nitrates. The following is a sketch of the reaction pathways described by O'Brien et al. (1975):

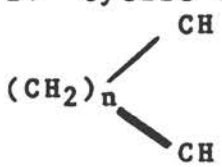


Many secondary organic aerosol compounds that have been identified in the atmosphere have been suspected to comprise a significant portion of the urban fine particle (D_{50} = < 2.5 μm) fraction in photochemical smog.

In the Pasadena California Air Characterization Study (ACHEX), Hidy et al. (1980) observed a number of secondary organic aerosol compounds, as presented in Table 4. Of course, these are not all of the possible compounds, but the list gives an idea of the complexity of the organic-aerosol generation process in photochemical smog. Size-distribution measurements completed during this study showed that a significant fraction of these aerosols had aerodynamic diameters of less than 0.5 μm. Unfortunately, there is apparently no direct relationship

TABLE 4

Secondary Organic Aerosols[a]

Compounds Identified	Possible Gas-Phase Hydrocarbon Precursors
Aliphatic multifunctional compounds	
1. X-$(CH_2)_n$ - Y(n = 3,4,5)	1. Cyclic olefins 
X Y	
COOH CH_2OH	
COOH COH	
COOH COOH	
COOH CH_2ONO	
or[b] COH CH_2ONO_2	
COH CH_2OH	
COH COH	and/or diolefins
COOH COONO	
or[b] COH $COONO_2$	>C=CH-$(CH_2)_n$-CH=C<
COH COONO	
COOH $COONO_2$	
COOH CH_2ONO_2	
2. Others:	2. Not known; possibly from aromatic ring cleavage
CH_2OH-CH=C(COOH)-CHO	
CH_2OH-CH_2-CH=C(COOH)-CHO	
CHO-CH=CH-CH(CH_3)CHO	
CH_2OH-CH=CH-CH=C(CH_3)CHO	
$C_5H_8O_3$ isomers[b]	
Nitrocresols	
$C_6H_6O_2$ isomers[b]	
Aromatic monofunctional compounds	
3. C_6H_5-$(CH_2)_n$-COOH(n=0,1,2,3)	3. Alkenylbenzenes C_6H_5-(CH_2)n-CH=CHR; also toluene for C_6H_5COOH
4. C_6H_5-CH_2OH	4. Toluene, styrene, other monoalkylbenzenes?
C_6H_5CHO	
Hydroxynitrobenzyl alcohol	
Terpene-derived oxygenates	
5. Pinonic acid	5. α-Pinene
Pinic acid	
Norpinonic acid	
6. Isomers of pinonic acid:[b]	6. Other terpenes?
$C_9H_{14}O_2$ isomers	
$C_{10}H_{14}O_3$ isomers	
$C_{10}H_{16}O_2$ isomers	

[a]Compounds identified at West Covina, California, 24 July 1974. (From National Academy of Sciences, 1976)
[b]Isomers not resolved by mass spectrometry.

between gas-phase photochemical reactivity of the various precursors and the quantity of aerosol formed. It is probably a complex function of the rate of reaction of ozone, oxygen atoms, and hydroxyl radicals, hydroperoxy radicals, and other radicals. In addition, the nature of a given hydrocarbon present will be of significance in determining how easily an aerosol is formed. This is related to the nature of the products formed, the product volatility, and the aerosol-formation-ability index. Given all these facts concerning the role of photochemistry in the production of photochemical smog, however, ozone accumulation downwind appears to occur in areas devoid of major sources of hydrocarbons and nitrogen oxides (Dimitriades and Altshuller, 1977). Some of the possible explanations include transport from urban areas, local generation of urban ozone precursors, local generation of ozone from anthropogenic and nature precursors, and injection of stratospheric ozone.

What are the conditions for chemical-chemical interactions in the atmosphere when photochemistry cannot occur, i.e., at night? The material produced by photochemical reactions during the daytime mixes to great heights in the troposphere (assuming a mean mixing height of 1500 m). In the evening, a portion of this material is cut off from the nocturnal inversion produced near the ground. Thus, ozone and other smog constituents are removed from the surface scavenging reactions and can persist for longer periods of time. A good demonstration of this

process was provided by the flight of DaVinci II on 8-9 June 1976, as shown in Figure 4 (Ripperton et al., 1976).

The DaVinci II experiment involved a surface-operated mobile van and a balloon, which moved along with the wind at an elevation of approximately 750 m. The balloon and van recorded ozone concentration, which increased to approximately 0.13 ppm in the midafternoon when the photochemical generation cycle was active. Overnight, the surface ozone was depleted, but the ozone aloft decreased only slightly. Therefore, many of the reactants and products associated with daytime generation processes remained in the air and were available for further reaction or enhancement of concentration during the next day.

Anderson (1978) proposed a model for nighttime chemistry (see Table 5). The results for an ozone, nitrogen dioxide, and propylene (C_3H_6) system show continued buildup of nitric acid (HNO_3), formaldehyde (CH_2O), and acetaldehyde (CH_3CHO); no sulfur dioxide was added to the reaction scheme. The ozone half-life determined according to this scheme was between 16 and 100 hours, depending upon the initial concentrations in the analyses. These are within the range previously indicated by field measurement for atmospheric ozone. Thus, ozone also would be available the next morning as an added "precursor" for any photochemical smog formation to occur the following day.

The various products of photochemical smog are also involved in the chemistry of sulfuric acid formation. These

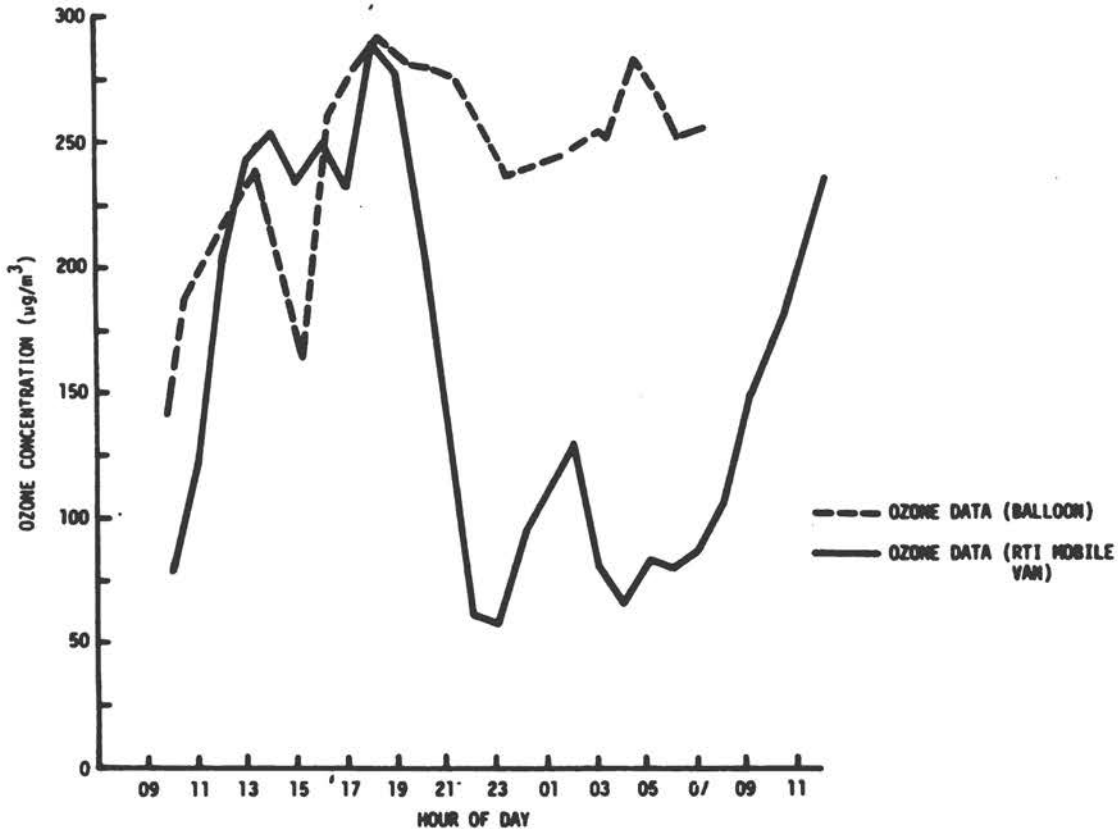


FIGURE 4. Airborne and ground-level ozone concentrations during the flight of DaVinci II on 8-9 June 1976. (From Ripperton et al., 1976)

TABLE 5

Mechanism for Nighttime Chemistry[a]

Reactions		k (x min)
1) $NO + O_3 \rightarrow NO_2 + O_2$		2.1×10^{1} ppm^{-1}
2) $O_3 + C_3H_6 \rightarrow HO + HCO_3 + CH_3CHO$	(net)	8.0×10^{-3} ppm^{-1}
3) $O_3 + C_3H_6 \rightarrow HO + CH_3CO_3 + CH_2O$	(net)	9.6×10^{-3} ppm^{-1}
4) $HCO_3 + NO_2 \rightarrow NO_3 + HO_2 + CO_2$	(net)	2.2×10^{1} ppm^{-1}
5) $CH_3CO_3 + NO_2 \rightarrow CH_3CO_3NO_2$		2.2×10^{1} ppm^{-1}
6) $CH_3CO_3 + HO_2 \rightarrow CH_3CO_3H + O_2$		5.3×10^{2} ppm^{-1}
7) $CH_3CO_3 + CH_3CO_3 \rightarrow 2CH_3OO + O_2 + 2CO_2$		3.2×10^{2} ppm^{-1}
8) $CH_3OO + CH_3OO \rightarrow 2CH_3O + O_2$		3.2×10^{2} ppm^{-1}
9) $CH_3OO + HO_2 \rightarrow CH_3OOH + O_2$		3.2×10^{2} ppm^{-1}
10) $CH_3O + O_2 \rightarrow CH_2O + HO_2$		4.8×10^{3}
11) $CH_3O + NO_2 \rightarrow CH_3ONO_2$		4.9×10^{2} ppm^{-1}
12) $OH + C_3H_6 + O_2 \rightarrow CH_3CHOOCH_2OH$	(net)	3.7×10^{4} ppm^{-1}
13) $2CH_3CHOOCH_2OH \rightarrow 2CH_3CHOCH_2OH + O_2$		3.2×10^{2} ppm^{-1}
14) $CH_3CHOOCH_2OH + HO_2 \rightarrow CH_3CHOHOCH_2OH$		3.2×10^{2} ppm^{-1}
15) $CH_3CHOCH_2OH \rightarrow CH_3CHO + CH_2O + HO_2$	(net)	1.5×10^{3}
16) $HO_2 + HO_2 \rightarrow HOOH + O_2$		5.3×10^{3} ppm^{-1}
17) $HO + NO_2 + M \rightarrow HNO_3$		1.1×10^{4} ppm^{-1}
18) $HO + CH_2O \rightarrow H_2O + HO_2 + CO$	(net)	2.2×10^{4} ppm^{-1}
19) $HO + CH_3CHO \rightarrow H_2O + CH_3CO_3$	(net)	2.2×10^{4} ppm^{-1}
20) $NO_2 + O_3 \rightarrow NO_3 + O_2$		4.6×10^{-2} ppm^{-1}
21) $NO_2 + NO_3 \rightarrow N_2O_5$		5.6×10^{3} ppm^{-1}
22) $N_2O_5 \rightarrow NO_2 + NO_3$		1.5×10^{1}
23) $N_2O_5 + H_2O \rightarrow 2HNO_3$		5.0×10^{-6} ppm^{-1}
24) $HO + CO \rightarrow HO_2 + CO_2$	(net)	4.4×10^{2} ppm^{-1}

[a]All calculations assume a constant concentration for carbon monoxide of 0.85 ppm and water of 1×10^{4} ppm (approximately 30% relative humidity). The sensitivity of the calculations to the rate constant for nitric acid formation (reaction 23) was tested using a fast value of 2.5×10^{-3} ppm^{-1} min^{-1} and the value used in this model of 5×10^{-6} ppm^{-1} min^{-1}. No significant difference was observed after 600 minutes. See text for explanation. (From Anderson, 1978)

aerosols are produced from the oxidation of sulfur dioxide, which usually persists for days in the atmosphere since summertime sulfur dioxide is emitted primarily from elevated sources and is transported downwind along with the other stable and free radical species. As shown in Figure 5, sulfur dioxide can remain in the air for several days and travel hundreds of kilometers downwind from its sources.

Furthermore, sulfur dioxide is the only sulfur oxide present in significant concentrations as a vapor. When it is oxidized to sulfur trioxide, which has a high affinity for water vapor, there is a prompt reaction to form sulfuric acid. The molecular-sized droplets of sulfuric acid are hygroscopic and will take an additional water vapor. Moreover, they will almost always be present in such high number concentrations that they will rapidly coagulate, forming fewer but larger droplets. Freshly formed sulfuric acid aerosol in the atmosphere will have median droplet diameters of 0.03 to 0.04 μm, but coagulation shifts the volume median diameter into the relatively stable accumulation mode (0.2 to 0.5 μm) within about 15 minutes. There is very little further change in particle size as the aerosol ages and reacts with ammonia .

OXIDATION OF SULFUR DIOXIDE

Oxidation of sulfur dioxide can take place as a gas-phase reaction, as an aqueous reaction after dissolution in a droplet, or as a reaction on the surface of a solid particle.

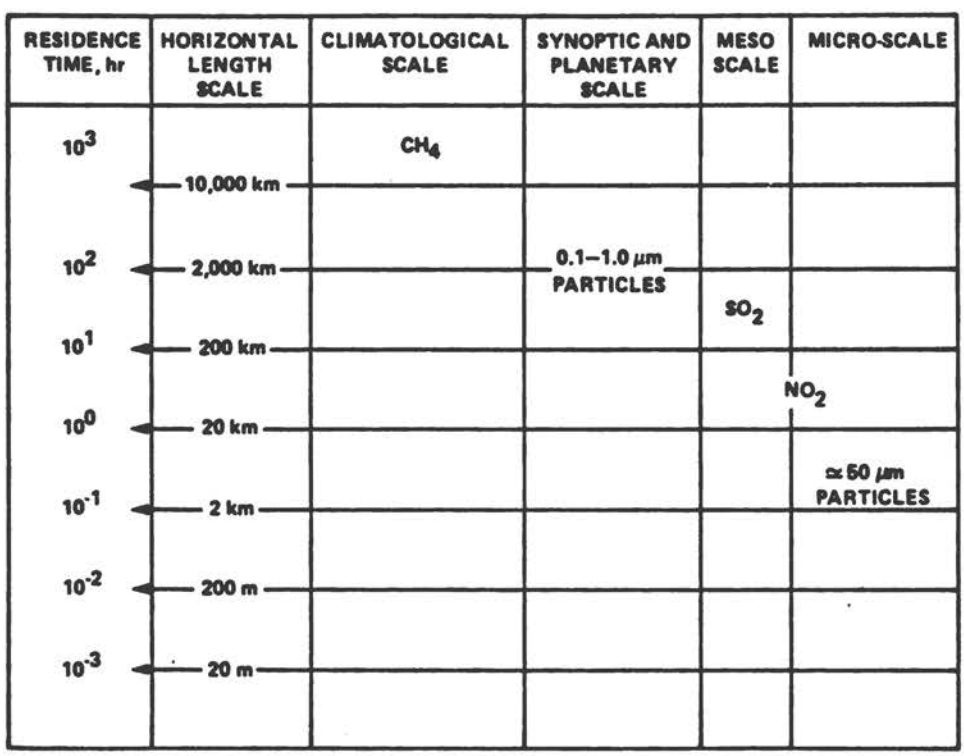


FIGURE 5. Estimated residence times for select pollutant species and their associated horizontal transport scale. (Air Quality Criteria for Particulate Matter and Sulfur Oxides, U.S. Environmental Protection Agency, 1981)

Gas-Phase Chemical Reactions of Sulfur Dioxide

Homogeneous gas-phase reactions have been most extensively studied and are better understood than any of the others. The U.S.Environmental Protection Agency (1981) recently summarized their pathways and rates. Calvert et al. (1978) systematically examined the rate constants and significance of elementary reactions of sulfur dioxide in the troposphere and concluded that many of the reactions were generally unimportant. These included: photodissociation, photoexcitation, reaction with singlet oxygen [(O_2 ($^1\Delta g$)], reaction with oxygen atom [$O(^3P)$], reaction with ozone, reaction with nitrogen oxides, reaction with tert-butylperoxy radical [$(CH_3)_3CO_2$], and reaction with acetylperoxy radical ($RCOO_2$). The only "important" sulfur dioxide reactions in the troposphere were those involving the hydroxyl radical, hydroperoxy radical, and methoxy radical. Table 6 lists the rate constants recommended by Calvert et al. (1978) for these three reactions, as well as the different rate constants for the hydroperoxy radical and the methoxy radical reported more recently by Graham et al. (1979), Burrows et al. (1979), and Sander and Watson (1981). The reasons for the discrepancies among these rate constants are unkown.

Although the dark reaction of sulfur dioxide plus ozone is too slow to be important in the troposphere, the addition of alkenes greatly enhances the oxidation rate. Calvert et al. (1978) reviewed and reevaluated the experimental work of Cox