United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington DC 20460

Research and Development

EPA/600/S8-85/020 Aug. 1986

EPA

Project Summary

Evaluation of Methods for Analysis of Human Fat, Skin, Nails, Hair, Blood, Urine and Breath

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This research program surveyed and evaluated the methods and procedures used to identify and quantitate chemical constituents in human tissues and fluids including fat, skin, nails, hair, blood, urine, and breath. These methods have been evaluated to determine their ease and rapidity, as well as cost, accuracy, and precision. During this evaluation, a second goal was to determine the feasibility of correlating a preferred method with a specific tissue/fluid and with easily identifiable chemical and physical characteristics of the analyte.

Because of these goals, the search strategy, as well as the literature evaluation focussed on analytical methods. The literature search was restricted to lists of chemicals of current interest to the U.S. Environmental Protection Agency, to references cited in "Chemicals Identified in Human Biological Media, A Data Base," and the "Chemical Abstracts Data Base." The information retrieved was summarized and classified by sampling and analysis methodology.

This Project Summary was developed by EPA's Office of Health and Environmental Assessment, Washington, DC, to announce key findings of the research project that is fully documented in two separate reports (see Project Report ordering information at back).

Introduction

For a long time, air monitoring constituted the major means of assessing workers' exposure to chemicals in industry. But this monitoring takes into account only exposure via the pulmonary route and, even for respirable chemicals, it does not indicate the actual uptake by the exposed worker.

Biological monitoring, the routine analysis of human tissues or excreta for direct or indirect evidence of exposure to chemical substances, has also been used to learn more about early detection of health impairment due to industrial chemicals. The types of analyses include the following measurements:

- concentration of the chemical in various biological media such as blood, urine, tissue and hair;
- concentration of metabolites of the original chemical in the same media; and
- determination of adverse/nonadverse biological changes of the organism resulting from exposure to reaction of the organism to exposure.

Hence, biological monitoring is used in the assessment of human exposure. A main goal of such monitoring is to ensure that the current or past levels of worker exposure are safe. Biological monitoring is being applied increas-

ingly to the assessment of exposure in environmentally exposed nonworker populations.

The purpose of this research program was to survey and evaluate the methods and procedures used to identify and quantitate chemical constituents in human tissues and fluids including fat, skin, nails, hair, blood, urine, and breath. These methods have been evaluated to determine their ease and rapidity, as well as cost, accuracy, and precision. During this evaluation, a second goal was to determine the feasibility of correlating a preferred method with a specific tissue/fluid and with easily identifiable chemical characteristics, such as octanol-water partition coefficient, water solubility, vapor pressure, etc.

Because of these goals, the search strategy and the literature evaluation focussed on analytical methods. The literature search was restricted to lists of chemicals of current interest to the U.S. Environmental Protection Agency (EPA), to references cited in "Chemicals Identified in Human Biological Media, A Date Base" up to 1982 and the "Chemical Abstracts Data Base." The Chemical Abstracts Data Base covers the complete chemical literature from 1967 to present, however, the search performed for this review was restricted to 1976 to 1983.

The list of target chemicals included in this review was compiled from a set of five documents received from the EPA Technical Project Monitor. The documents contained lists of compounds of interest to the EPA.

The chemicals were classified into a hierarchial scheme based on their physical and chemical properties. This scheme first separated inorganic from organic compounds. Organic chemicals were then separated according to functional group substituents. Functional groups that have the greatest effect on a compound's physical and chemical properties and/or analytical behavior are placed highest in the scheme. For example, carboxylic acids are ranked above acid esters. The acid moiety is a stronger functional group with a greater influence on aqueous solubility, boiling point, extraction behavior, and chromatographic properties. If a compound contains more than one functional group, it is placed in the highest chemical classification. Because this classification is based on the physical/chemical properties, it should reflect analytical behavior of many of the compounds to aid in the overall evaluation process.

Information from the data base "Chemicals Identified in Human Biological Media" on (1) literature referenced, (2) chemicals analyzed, (3) biological matrix, and (4) analytical technique was entered and stored on computer files. These data were retrieved according to analytical method. Under each analytical method, citations referring to that method were listed according to chemical class, specific chemical, and biological matrix. The "Chemical Abstract Data Base" was searched using the selected compounds which were cross-referenced by CAS number with the human biological matrices of interest. This information was used for sorting retrieved literature citations and disseminating articles to members of the advisory committee who were responsible for the preparation of this review. This information was also used to match analytes with biological matrix in order to determine the feasibility of correlating analytical method with biological matrix and with chemical characteristics.

Relevant articles which report the use of an analytical technique to measure any of the target chemicals in the seven biological matrices of interest were obtained. Only those analytical methods which had at least three articles cited were included in the review.

During acquisition of literature citations, more than 95% of all citations identified as relevant, i.e., those which reported measuring any of the target chemicals in the seven biological matrices, were retrieved. Missing citations were not retrieved for several reasons:

- the article was in a foreign language which could not be translated easily;
- the reference in the data base was incorrect; or
- a source of the article could not be located.

The full report was written to evaluate each analytical method. The evaluation for each method included sections on instrumentation and sample preparation methods. Much of the information on analytical instrumentation was taken from review articles, reference texts, or our working knowledge of the techniques. Information on sample preparation methods were generally restricted to the citations in the data bases. The section on method/analyte correlation parameters attempted to suggest physical or chemical properties of analytes which could be used to predict the applicability of the given method to the analysis of that chemical in a specific biological matrix.

Conclusions

Methods and procedures that were cited in both data bases for the identification and quantitation of the target chemicals in the seven human biological matrices were summarized. Broad generalizations are difficult to formulate due to the number of compounds, complexity of matrices, and the variety of methods reviewed. A noncritical survey of the analytes for each method in the data base with a brief summary of the advantages and disadvantages of each of the methods reviewed follows.

Atomic Absorption Spectrophotometry

Atomic absorption spectrophotometry has been the method of choice for many investigators who require a highly specific, low cost technique for elemental analysis with ultratrace detection limits. The advantages of this single-element method have been known for the past 15 years, but the use of modern instrumentation has produced analytical data of greater reproducibility and has resulted in increased sample throughput. One of the best documented environmental measurements (lead in whole blood) is usually performed by atomic absorption methods.

The biggest disadvantage of this technique is not the inherent limitations of the method with respect to analyte specificity and sensitivity, but problems with matrix effects. A variety of digestion conditions have been developed which in combination with deuterium background correction and/or chelation-extraction, such as APDC MIBK, have been successful in reducing matrix effects and form the basis for most atomic absorption procedures.

Neutron Activation Analysis (NAA)

Neutron activation allows multielement analyses on a single sample without pretreatment. Under conditions of high neutron flux, maximum sample size (approximately 1 g) and no interferences, NAA provides detection limits which can only be matched by flameless atomic absorption spectrophot

metry. Essentially all of the elements of environmental interest (with the exception of beryllium and lead) from nitrogen to the heavy elements can be analyzed simultaneously by this technique.

The principal limiting factor in neutron activation analyses is the presence of interferences that result in the gamma-ray signal from the various sources not being resolved. Detection limits may increase by several orders of magnitude when interferences are present. Poorer precision and accuracy also result.

Accessibility to a neutron source notwithstanding, NAA can be relatively simple and inexpensive with the capability of measuring a large number of elements simultaneously. When either chemical methods or extensive time resolution is required to minimize interferences; the cost, time, and labor required for analysis increase significantly.

Electrochemical Methods

The facility of ion selective potentiometry in the analysis of inorganic ions is a distinct advantage of this analytical technique. Although limits of detection are typically 10^{-6} *M*, which is somewhat higher than competing techniques, fluoride and cyanide selective electrodes have been quite successfully used in the analysis of blood and urine. Their simplicity in this regard makes them highly recommended.

Anodic stripping voltammetry (ASV) has been applied to the analysis of blood, urine, and hair for a large number of metals including zinc, cadmium, lead, chromium, and copper. Since ASV is capable of differentiating between labile and nonlabile metal species, it is clearly the method of choice when information regarding metal speciation is desirable. When total metal determinations are needed, ASV is comparable to other methods with respect to labor required and accuracy.

Cyclic voltammetry has been applied to several metal determinations and, in addition, some organic compounds such as p-amino-diphenylamine. Cyclic voltammetry is an easy technique to implement and the analysis is fast; blood samples have been analyzed without further treatment. The equipment cost is relatively inexpensive compared to other instruments used in the simultaneous determination of metals. The limit of detection (LOD) is higher than for ASV.

Emission Spectroscopy

A large number of metals may be determined using emission spectroscopy. The limit of detection for these metals is adequate for environmental trace analysis, and many published data were found on the use of either DC or argon plasma ionization methods. However, by far, the most widely used technique is atomic absorption (AA) spectroscopy. Because of the wealth of data on method performance and the availability of instrumentation, AA would be considered the method of first choice, but ICP may be faster, more accurate, more precise and have lower LOD.

Proton Induced X-Ray Emission (PIXE)

Proton induced X-ray analysis may be used for multielement analysis of a large number of samples. Although biological samples can be analyzed in a variety of forms, including untreated specimens, best results are achieved by digesting the organic material and placing the resulting solution on a Mylar film for irradiation. The elements normally measured for PIXE range from phosphorous ($z = 15$) to lead ($z = 82$) with limits of detection at the subnanogram level.

The method is simple, allows high sample throughput, and is currently being performed inexpensively by several laboratories.

Spectrophotometry (Colorimetry)

Spectrophotometry has the advantage of being a simple and quick procedure utilizing instrumentation which is widely available and relatively inexpensive. It is capable of determining compounds and metal complexes at the low microgram to high nanogram range with good precision (2-3%). The disadvantages of this technique are the labor intensive sample preparation steps, and the nonunique nature of the measured signal (compared to AA/ICP). This method is suitable for many analytical measurements, particularly where the operating laboratory does not have AA/ICP spectrometers. When available, AA or ICAP would usually be the methods of choice.

Gas Chromatography

Gas chromatography is used for the separation and determination of volatile or semivolatile organic compounds. Recent developments in capillary column chromatography provide for very high resolution to give separation of complex samples. The use of specific column packings allows the determination of polar and some compounds with low volatility.

Effluent from the GC column may be analyzed using a specific (e.g., electron capture, nitrogen/phosphorus) or a nonspecific (e.g., flame ionization, thermal conductivity) detector. Limits of detection range from subpicogram to microgram depending upon the detector used and the compounds determined.

Gas chromatography is a simple technique for the determination of organic compounds and is used extensively on human biological extracts. Compounds which are nonvolatile (B.P. >300°C), very polar, or heat labile cannot be determined directly by this method. For these compounds, high performance liquid chromatography is generally used. Some polar and/or nonvolatile compounds may be derivatized to improve gas chromatographic performance.

Most biological samples cannot be analyzed directly. Analytes are usually extracted and fractionated prior to analysis. Although GC analysis itself is a simple rapid procedure, sample preparation techniques are often complicated and time-consuming.

Mass Spectrometry

Mass spectrometry is a sensitive, specific but expensive technique. The various forms of mass spectrometry that can be employed complicate the question of when the technique should be used. Spark source mass spectrometry is extremely valuable in elemental analysis. Atomic absorption spectrophotometers and inductively coupled argon plasma spectrometers are substantially less expensive for these analyses; however, for a semiquantitative scan of a broad range of elements, spark source mass spectrometry could be the method of choice. Many commercial laboratories offer spark source mass spectrometric assays as a service, so purchase would not be necessary for an occasional need. The main use of mass spectroscopy is in the identification and quantitation of organic compounds.

Isotope ratio mass spectrometry is the preferred method for accurate determination of isotope ratios. If such an assay is required, specialized laboratories make effective use of the instrumentation.

Although gas chromatography/mass spectrometry as a combined technique is one of the most widely used analytical methods, the decision to use the technique should be evaluated carefully. Gas chromatography is far more cost-effective and is usually more sensitive. However, the analysis of biological samples for various organic constituents by gas chromatography presents various difficulties which may require the specificity inherent in the mass spectrometric technique. For example, if identification of a pesticide is desired for forensic or regulatory purposes, the use of gas chromatography/mass spectrometry with authentic standards will give the needed information. Biological samples often contain material which coelutes with a compound of interest and only the specificity of the mass spectrometric technique will give a successful assay. When standards for the compounds of interest are not available, identification can be carried out with a high degree of confidence only by the mass spectrometric technique.

In summary, mass spectrometry should be used when its specificity is required, a less expensive technique is not adequate, or when certain types of information are available from no other source, for example, accurate isotope ratios or accurate mass measurement.

X-Ray Spectroscopy

X-ray emission spectroscopy can be used in chemical analysis for both quantitative and qualitative studies. X-ray spectroscopy is applicable to the determination of most metals in multielement analysis. The technique is expensive and not widely available.

Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance spectroscopy is applicable to a wide range of organic compounds, provides information on chemical structure, and can be quantified. The operation of NMR instruments is rather complex; however, no significant sample preparation is required. The equipment is very expensive and not widely available.

Thin Layer Chromatography

Thin layer chromatography is a separation technique based on an adsorption or partition process and employs a thin, flat bed of sorbent. Thin layer chromatography can be used for the separation and semi-quantitation of complex mixtures and has proven useful as a cleanup technique for analytes present in biological fluid extracts and environmental samples.

With the exception of some macromolecules, reactive compounds, and volatile substances; virtually any organic compound and many inorganic ions can be detected by thin layer chromatography. The limit of detection is a function of the specific detection system utilized. Semi-quantitative thin layer chromatography is fast, simple and can be accomplished with a minimum of expensive equipment. Quantitative thin layer chromatography with modern instrumental detection systems is both accurate and sensitive but requires rather expensive equipment.

High Performance Liquid Chromatography

High performance liquid chromatography is a simple and standard separation technique applicable to virtually any mixture of organic compounds as well as certain ionic species. It is usually applied to complex matrices for which gas chromatography is not usable, such as matrices containing high boiling point chemicals or compounds which decompose under GC conditions. A wide variety of detectors can be interfaced to a liquid chromatography column such as ultraviolet detectors, refractive index and fluorimetric detectors.

Miscellaneous Methods

During this survey, two reports were found dealing with the determination of compounds of interest using some miscellaneous methods. The scarcity of publications did not warrant a special section for these techniques in this review.

One such report describes the evaluation of the permeability of phenolic compounds through human stratum corneum using a desorption technique. In another report, laser microprobe mass analysis (LAMMA) is used to determine metal ions and organic compounds in solid samples which require spacial resolution.

Methods for Breath Analysis

Several combinations of sampling, concentration, and analysis methods were cited in the reviewed literature for the identification and quantitation of target compounds in human breath. A brief noncritical review of the advantages and disadvantages of each method follows.

In 1977 an international workshop on "The Use of Biological Specimens for the Assessment of Human Exposure to Environmental Pollutants" summarized the data on the location or compartmentalization of xenobiotics and/or their metabolites in various biological matrices including breath. They concluded breath is not useful to evaluate exposure to arsenic, beryllium, cadmium chromium, fluorine, lead, mercury molybdenum, manganese, nickel, vanadium, zinc, copper, cobalt, platinum palladium, tin, methyl mercury, DDT phenoxy herbicides, pentachlorophenol, PCB, PBB, PCN, tetrachloro dibenzo-p-dioxins, benzofuran, azobenzene, polycyclic hydrocarbons, amine and nitro derivatives, organophosphorus esters, cyanides, nitriles, mycotoxins, antibiotics, and hormonal substances. They concluded breath levels correlate with body burden in the case of chlorinated solvents, plastic monomers, fluorinated propellants nonsubstituted aliphatic and aromatic volatile hydrocarbons, alcohols, ethers ketones, and carbon monoxide. It was also concluded selenium breath level could possibly be useful as an indicator specimen but further research was required to confirm selenium.

Apnea Direct Injection/Gas Chromatography

The apnea sampling method combined with direct injection of the expired air into a gas chromatograph is an extremely simple and fast method which requires no specialized equipment. Since no concentration is achieved however, the limit of detection is relatively high for this method.

Breathing Valve/Direct Injection/Gas Chromatography

This method, like the apnea/direct injection/GC method, is simple and fast. A breathing valve, air tank, and sample collection bag are required making it slightly more complicated than the apnea method. Again, the sample is not concentrated before injection into the gas chromatograph, resulting in relatively high limits of detection. Because very little sample manipulation is performed, recoveries are high. Specific recovery values were not found in the literature reviewed.

Breathing Valve/Adsorbent Concentration/Gas Chromatography

This method is complicated and expensive compared to the direct injection techniques. The concentration achieved, however, results in very low detection limits (as low as 1 part per trillion). This allows a much larger number of trace components to be identified and quantitated, but also leads to more problems with background interferences. Some reported recoveries are high, better than 95%, with coefficients of variation of 3-6%.

Cryogenic Trapping/Gas Chromatography

This method concentrates the compounds of interest without concentrating the oxygen and nitrogen matrix. The extended sampling period (60 minutes) results in the equivalent of sub-ppb detection limits although contaminants in the supply air could increase the limit of detection for those compounds. The cryogenic trapping method is sensitive but also complicated, time-consuming, and requires a specialized cryogenic trapping system. No recovery data are available for this method.

Tenax GC Cartridge/GC/Mass Spectrometry

This method concentrates the compounds of interest by directly adsorbing the breath or breath collected in a Tedlar bag onto a Tenax cartridge. The Tenax cartridge is later thermally desorbed and injected into a GC/mass spectrometer. This method has excellent sensitivity but it is costly and requires elaborate equipment. The percent recovery for laboratory samples ranges from 77 to 110 percent.

Cascade Impactor Sampling/ Photon Induced X-Ray Emission (PIXE) Analysis

This is a sensitive but very complicated method for elemental analysis of breath. Exhaled air (4-6 L) is concentrated for PIXE analysis using a cascade impactor which results in detection limits at the nanogram level. Sample collection efficiency is high (85-99%) which should lead to high recoveries. The disadvantage of this technique is that a large amount of specialized and expensive equipment is required including cascade impactors, a computer for spectral analysis, and a Van de Graff accelerator for PIXE analysis.

Recommendations

The goal of this research program was to evaluate methodologies for determining chemicals of interest to EPA in human biological matrices. This review included the citations listed in "Chemicals Identified in Human Biological Matrices, A Data Base" and the Chemical Abstracts Data Base. Based on this extensive literature, summary reviews have been compiled according to methodology and form the bulk of the full report.

In addition, based on the chemical and physical characteristics of the compounds of interest and the method by which they are determined, some simple and expected correlations can be found.

Some general recommendations can be summarized as follows:

1. Many volatile and semivolatile organic compounds found in human biological matrices can be determined by gas chromatography coupled with a variety of detectors. To select a specific detector, the compound type and the matrix type being analyzed must be taken into account.
2. Organic compounds of high boiling point or that decompose under gas chromatographic conditions can be determined by liquid chromatography coupled with a variety of detectors. Again the selection of a specific detector depends on the compound and matrix type being analyzed.
3. Adsorption onto Tenax cartridges is the most widely used breath sampling method to preconcentrate organic compounds, including hydrocarbons, chlorinated hydrocarbons, alcohols, ketones, nitrogen-containing compounds and others.
4. Most metals and metal-containing compounds can be analyzed by atomic absorption spectroscopy or inductively coupled argon plasma. When the metals are in solid matrices such as hair and tissue, they can be determined by neutron activation analysis and proton induced X-ray spectroscopy without further sample preparation. Some specific metal matrix combinations may be best analyzed by electrochemical methods or colorimetric methods. Other techniques applicable to the analysis of metals and organometallic compounds were found, however, they do not appear to be as widespread and convenient as the ones mentioned above. With the exception of lithium and beryllium, most metals in breath can be determined by PIXE analytical techniques.

Body Burden

Biological monitoring of humans for xenobiotic body burden entails the collection and analysis of biological specimens (organs, tissues, blood, etc.).

Biological monitoring is useful for several reasons. First and foremost, it provides direct evidence of exposure and absorption of a xenobiotic. It thus alerts one to the possibility of a human health hazard. The data obtained by biological monitoring can be correlated with the observed level of pollutants in the environment and with the incidence of human diseases, which may be due in part to the presence of the pollutants. These correlations can be used to determine priorities with regard to research relating to human health and to determine the necessity of regulation of pollution sources. Biological monitoring can point out pollutant problems which are not otherwise noted, such as could occur due to bioaccumulation of pollutants in humans resulting from long-term exposure to very low levels of pollutants in the environment.

In 1977 an international workshop was held in Luxembourg on "The Use of Biological Specimens for the Assessment of Human Exposure to Environmental Pollutants." The objectives of the workshop were:

(a) To assess the types of environmental pollutants and human specimens most suitable for biological monitoring and to evaluate the probable usefulness of biological specimen banking.
(b) To examine the state of the art and technical feasibility of programs designed to collect human biological specimens for biological monitoring and biological specimen banking.
(c) To develop guidelines on sampling, sample preparation, storage, and analytical requirements.
(d) To make recommendations for further research and development.

Exposure routes for organic chemicals are usually inhalation, ingestion, and dermal absorption. Once absorbed, the chemical may be excreted, (includ-

ing exhalation) stored, or metabolized. In general, chemicals are metabolized to a more polar form which leads to conjugation and excretion in the urine. The fate of any chemical will depend upon its volatility, polarity, and chemical and biological stability. For example, polychlorinated biphenyls (PCB) and polychlorinated terphenyls (PCT) are nonvolatile, inert, lipophilic compounds which are stored in the adipose tissue. More polar compounds such as phenols and acids are generally excreted in the urine.

Several reports found in the published literature described the simultaneous analysis of many elements in human biological matrices.

Significant differences in the trace levels of chlorine, potassium, calcium, titanium, manganese, iron, copper, and lead in hair from different parts of the body were found. This suggested that the trace elemental absorption and accumulation in hair not only depends on the particular element, but also on the location of the hair in the body. The trace concentrations in white and black hair was also found different.

Seventeen different elements were analyzed simultaneously in healthy and pathological tissue to obtain information on the cancerous process. Significant differences in the content of various elements were found in the normal and pathological tissue. Potassium, zinc, and selenium were found in higher concentration in the cancerous mucosa of the stomach. No significant differences appeared in the elemental composition of blood, erythrocytes, hair, and striated muscle taken from the abdominal wall from patients with gall bladder or stomach cancer when compared with other noncancer diseases.

Human hair root has been analyzed for the presence of several elements. Hair root, rather than strand may reflect the most recent exposure influences and its analysis is exclusive of externally acquired constituents. Samples collected from 23 randomly selected rural Florida children, three to six years of age were analyzed. The results showed differences in the Fe content by sex and Cu (and possibly others) differences caused by intake.

Human blood serum was analyzed for zinc, copper, iron, chromium, manganese, and selenium as part of a survey to determine whether or not the Australian aboriginal people received optimal diet. Special attention was directed to chromium because of the high incidence of diabetes mellitus in this population.

Multiple-element analysis of human cerebrospinal fluid and other tissues was used to determine diseases of the nervous system. The distribution of copper, iron, and zinc in the cerebrospinal fluid was such that deficiencies could not be studied. For the majority of trace elements studied, the normal values could not be determined much less the deficiencies. The interpretation of sporadic high levels was not possible. High levels of silicon found were not anticipated. The accumulation of silicon in the body fluids of patients with renal failure and on dialysis coupled with the clinical correlations made silicon a candidate for an uremic neurotoxin. The higher levels of silicon in cerebrospinal fluid of clinically-diagnosed and autopsy-proven cases of Alzeheimer's disease coupled with the presence of silicon in the neurofibrillary tissue of these patients suggest a correlation of silicon levels with this poorly understood disease.

Twenty-four elements were measured in hair from 20 individuals who worked in a lead-zinc smelter in Poland. These same elements were also measured in hair taken from 20 individuals considered "normal" or not exposed. Elements found to be at elevated levels relative to the controls included As, Se, Ag, Cd, and Sb.

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***James Bridges** is the EPA Project Officer (see below).*

The Project Summary covers the following reports:

"Evaluation of Methods for Analysis of Human Fat, Skin Nails, Hair, Blood and Urine," (Order No. PB 85-242 790/AS; Cost: $28.95, subject to change)

"Methods for Sampling and Analysis of Breath," (Order No. PB 85-243 277/AS; Cost: $9.95, subject to change).

The above reports will be available only from:

National Technical Information Service
5285 Port Royal Road
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The EPA Project Officer can be contacted at:

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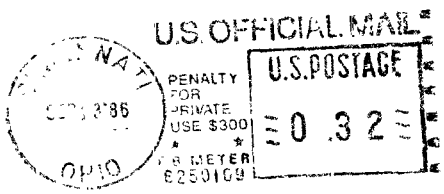

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