ANALYTICAL CHEMISTRY SYMPOSIA SERIES - volume 20

topics in forensic and analytical toxicology

Proceedings of the Annual European Meeting of the International Association of Forensic Toxicologists, Munich, August 21-25, 1983

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ELSEVIER
Amsterdam – Oxford – New York – Tokyo 1984

ELSEVIER SCIENCE PUBLISHERS B.V.
Molenwerf 1
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

Distributors for the United States and Canada:

ELSEVIER SCIENCE PUBLISHING COMPANY INC.
52, Vanderbilt Avenue
New York, NY 10017, U.S.A.

Library of Congress Cataloging in Publication Data

International Association of Forensic Toxicologists.
European Meeting (1983 : Munich, Germany)
Topics in forensic and analytical toxicology.

(Analytical chemistry symposia series ; v. 20)
Includes index.
1. Forensic toxicology--Congresses. 2. Poisons--Analysis--Congresses. I. Maes, R. A. A., (Robert A. A.), II. Title. III. Series.
RA1228.I575 1983 614'.1 84-1680
ISBN 0-444-42313-3 (U.S.)

ISBN 0-444-42313-3 (Vol. 20)
ISBN 0-444-41786-9 (Series)

Printed in The Netherlands

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edited by R.A.A. Maes

PREFACE

Nowadays, the forensic toxicologist has come far in our fast-moving society from the days of hemlock and arsenic to drugs and all kinds of environmental poisons.

The demand for analyses with a high degree of accuracy, sensitivity, precision and specificity has become acute in the face of modern analytical toxicology.

These analyses will provide typical data which will be used for the interpretation of case reports.

Profound analysis of collected data will contribute to the evaluation of future cases.

The Annual European Meeting of the International Association of Forensic Toxicologists was held in Munich in the Federal Republic of Germany on August 21-25, 1983, under the excellent presidentship of M. Geldmacher-von Mallinckrodt, Professor of Toxicology at the Institute of Legal Medicine in Erlangen - Nürnberg, F.R.G.

This congress covered many topics of analytical toxicology including related fields, e.g. drugs and driving.

Plenary sessions were keynoted by distinguished contributors and were followed by invited and selected papers from the participants.
Major topics covered included

- Drugs and driving
- Drugs of abuse
- Toxicological analysis of drugs and their metabolites, their clinical and forensic implications
- Mass spectrometry in toxicology
- Combination methodology in toxicological analysis
- Systematic toxicological analysis
- Toxicology in developing countries

Special attention was paid to round table discussions involving quality control and documentation, as well as post-graduate education in forensic toxicology.

These discussions were envisaged as an opportunity for meaningful exchanges of ideas and information among all toxicologists present, in an informal congenial atmosphere. I believe that this goal was achieved. It was hoped that in this setting the exchange of knowledge and experience in these fields would

lead ultimately to innovative approaches to problems in forensic toxicology.

It is a pleasure for me to express sincere appreciation to the authors who participated in the different scientific sessions that formed the basic structure for this meeting and whose papers are presented in full in this volume.

I'm grateful to the Staff of the Organizing Committee and of Elsevier Science Publishers B.V. for all the help they have given me in producing this book.

Robert A. A. Maes

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R.A.A. Maes (Editor), *Topics in Forensic and Analytical Toxicology*

Opening Lecture

TOXICOLOGY - PAST, PRESENT AND FUTURE

A.S. Curry, 24 Lima Court, Bath Road, Reading RG1 6NH, U.K.

It gives me great pleasure to give the Opening Lecture at this most important Meeting. The inauguration of the International Association of Forensic Toxicologists took place twenty years ago and its founders had a vision of International mutual co-operation which has succeeded and is being cemented here today in these magnificient surroundings. I wish to congratulate and thank the organising Committee and, in particular, the Countess Geldmacher von Mallinckrodt.

I have chosen to survey the past, present and, to a small extent, the future of forensic toxicology over a period of about thirty years, since it covers my personal experience and because over this period forensic toxicology has undergone similar advances to those dramatic ones in aviation - (London to New York in $2\frac{3}{4}$ hours) - and in space exploration - (a trip to Mars and the space shuttle).

In toxicology we have seen an increase in the sensitivity of detection of at least a million times over most of the range of poisons and this has been coupled with the explosion in compounds available to the public which have been mainly in the pharmaceutical and agricultural areas.

In discussing these changes I wish to submit that the rate of change has been so great that workers in the field have been very busy in keeping up to date negotiating to buy new instrumentation, learning how to use them, publishing some results and then moving onto the current year's "best buy".

I wish to comment on what I believe to be a reality now - that it is almost an impossibility in the vast majority of smaller laboratories nowadays to do a comprehensive search for "poisons".

I have always believed that the criminal poisoners are cool, calculating, criminals out to defeat the professional skills of the physician, pathologist and toxicologist.

In the 1950s a reasonably complete analytical search could be done - after all the "alkaloid" group consisted of, at the most, about 10 bases instead of over 500 now. Then, to detect morphine, nicotine, strychnine, atropine or aconitine, in a Stas-Otto extract of 500 grams of liver required a good eye for the colour tests, an acute sense of taste (for example, Sir Thomas Stevenson with aconite), and a great deal of imagination! The number of acid solvent soluble drugs was few - aspirin and the barbiturates predominated - now it involves nearly 200. As far as metals were concerned, provided one had looked for arsenic, antimony, mercury and lead the major poisons had been covered - most by a simple Reinsch test. Organo-phosphorus compounds did not exist, nor did fluoroacetate and thallium had not become what it is now called - "the social menace".

The literature concerning toxicological analysis was readable in a few evenings - it was written by real generalists like Orfila, Christison, Winter, Blyth, Autenreith and Bamford. These books should still be compulsory reading for students. Research papers published in the previous 50 years could be counted on one's fingers and toes and the use of chromatography only began to make its mark, firstly using paper, post 1953.

The information explosion has led us from card indices kept personally, through professional journals containing abstracts to the computer based retrieval systems in use today.

Computers now enable the rapid matching of spectra, be they infra-red, ultra violet, mass spectrographic or gas chromatographic, with data bank collections and in the literature field for the analyst to do a general search before he even looks at the viscera, get references to analytical methods, discover potential side effects of drugs or synergism between drugs and finally to compare his analytical results with those in the literature and, in the UK, with those from all his colleagues going back several years. This latter capability is most valuable in that when a drug new to the analyst is encountered, the computer may reveal that it has been met by a colleague in another laboratory who can then share his experience with the initiate. I am delighted to learn that TIAFT now has access to the Aldermaston data bank.

It is my thesis that this explosion has contributed to a fundamental change in philosophy over the last 30 years - now the emphasis is on looking for drugs known to have been available to the deceased. I cannot remember a paper in the last few years starting with the assumption "we don't know what we are looking for". One of the reasons for this has been the inability of one toxicologist to perform all

the tests himself - so one now has experts in gas chromatography, mass spectrometry, immunological methods, etc., with very few workers who know how to spend hours searching the gastro-intestinal tract, discovering perhaps the residues of amanita phalloides, or not finding the residues of a crushed up gramophone record! (This latter search, in a case in which I was involved, was occasioned by an allegation in a poison murder trial.) The normal glass fragment content of faeces was another unusual analytical investigation I found necessary in a case in which administration was alleged - I never knew, until then, that green glass on peas was used with homicidal intent in certain parts of the world.

These reminiscences, however, show how some techniques, involving only the eye, have not changed.

In the early 1950s the main instrumentation consisted of a sublimation (under reduced pressure), apparatus and also a device for doing mixed melting points, side by side with controls. In my first murder case, which involved phenobarbitone, I had to use an ultra-violet spectrophotometer - manual of course - in the nearest University. Special rooms were necessary to house the very large infra-red machines and it was not for several years that they became routine instruments in toxicological laboratories. En passant, let me recall that arc emission spectroscopy was borrowed from the forensic chemists and used to analyse the Group precipitates following a nitric/sulphuric acid digest of 100 grams of liver or kidney. I have spent many hours doing this digestion on each of many hundreds of cases - I cannot remember one where it revealed anything useful other than to confirm a previous suspicion.

One of the favourite poisons used for homicide in the 1950s came from rat and slug baits containing yellow phosphorus. This later went off the market being deemed to be cruel to rats! They were halcyon years for the toxicologist - in one case alone, in which I and Neville Dunnett were involved, 3 husbands and one lodger were exhumed. I do not think there is a more beautiful sight in toxicology than when one lets in the air on the first few seconds of distillation and the glorious phosphorescence screams the presence of yellow phosphorus. I suppose nowadays the defence would want a video-recording of it! It was the one poison that provided the perfect excuse to be in a darkened room with a female student! In mentioning exhumations, this is another area which has not been properly covered in the technical literature. I am sure it is as necessary as the latest report on the use of mass spectroscopy. We have been very fortunate in Europe in having the two foremost workers - Kaempe and Stevens - in the area of the production of unusual compounds as a result of putrefaction. Their work has been of great help

not only in the case of exhumations in temperate climates, but also in routine cases in countries where the heat is intense, it may take days for an autopsy to be arranged and weeks for the organs to reach the laboratory. Strong brine may be the only preservative available. I can assure you that in many parts of the world these conditions are usual - I have seen them myself - and I have the greatest admiration at the success rate of the toxicologists who have to work under such conditions.

To return to the analyses for organic solvent soluble poisons it was fortunate that in the 50s the toxic dose of the substances available to the man in the street resulted in many milligrams being present in the extracts from 100 grams of liver. It was an added bonus that the majority crystallised beautifully and in some laboratories X-Ray diffraction was available for ultimate identification. I don't think I ever managed to get seconal to crystallise but fortunately paper chromatography soon came to our aid.

I am sure that the use of paper chromatography for barbiturates, the alkaloids and to a smaller extent, the metals, represents the most significant advance in toxicology in the 1950s. With the separative properties of the technique, on a few microgram scale, was coupled the use of ultra-violet light at different pHs, as well as selective sprays and dips to reveal, with beautiful colours, functional groups and so were married the classical colour tests with the massive new separative capability of chromatography.

You will remember that in 1954 Harry Powell and I published the use of a butanol:citric acid solvent on citrated paper for the separation of the then common alkaloids. It is pleasing to see that it is third in its discriminating power of a very full survey done by Moffat using thin layer chromatography nearly 30 years later. I can't remember what made me try pouring concentrated sulphuric acid/formaldehyde (Marquis reagent) over the developed, dried, chromatogram - theory said the paper would blacken or go at least yellow - but low and behold the separation of codeine and morphine by nearly an Rf point was revealed and the blue/purple colour differences shouted their differences. All the interfering browns were now on the solvent front.

The detection with permanganate of unsaturated parts of molecules - for example in seconal - and of bromine using the fluorescein-eosin reaction to reveal bromobarbiturates are examples of tests that were developed using a rational chemical basis.

The use of 254 mμ light - using the change of absorption in ammonia vapour

and acid as well as the measurement of full uv spectra on eluted spots provided a most useful test for 5:5 disubstituted barbiturates and the full spectra of alkaloids was invaluable. The fluorescence of quinine and ergometrine have been most useful to me: the latter figured in a homicidal insulin poisoning in which I did the analyses and so high is the fluorescence that one was able to get down into the nanogram region of detection. This was essential to prove a negative. So in a few years, the melting point test using milligrams of sublimed poison had given way to rational chemistry on a few micrograms of organic solvent soluble poisons and occasionally on much less. One of the most esoteric tests I did was to use Wieland's classical work on the amanita toxins and show that injection from a paper chromatogram of an intestinal content extract, at the Rf of amanita, killed mice.

In the analysis of metals there was little progress although group reactions and such reagents as dithizone were useful in some cases. A lot of work was done in academic establishments in this area and many toxicologists tried out the techniques. I do not think it came in widely as a routine technique although I can well remember the beautiful green fluorescence of lead and several metals showed absorbent spots in 254 mμ light.

The ability to use tests which were much more sensitive meant that no longer was it necessary to use 100-500 grams of tissue in order to isolate a detectable amount of poison. Tungstic acid, aluminium chloride and hydrochloric acid came into the isolation vocabulary instead of Stas-Otto and ammonium sulphate; an analysis for barbiturate on stomach contents, blood and liver could easily be completed in 24 hours - 12 if one chose one's starting time. However, the pride of a much better job done in relation to the barbiturates quickly led to a demand which expected a 24 hour service for <u>all</u> poisons. The English have an expression "hoist by one's own petard"! This sociological phenomenon has been glossed over very largely to the toxicologist's own detriment. The authorities investigating death got into their minds that instant toxicology had arrived which was exacerbated by hospital laboratories now being able to do their own blood barbiturate analyses. The ability of the forensic toxicologist to analyse for say morphine in blood was similarly expected. Perhaps this was the spur that kept us burning the midnight oil and making our own gas chromatographs. How many remember the boiling toluene vapour jackets and hydrogen cylinders in the laboratory of the late 1950s? I mavel that we got away without major fires and explosions but gas chromatography was another technique which increased sensitivity of detection and speed of response. It also marked the first signs of emergence of the expert in a particular technique in toxicology. One person could do the isolation work while a paper chromatogram was running but it could take a morning's attention to warm up and stabilise an

early gas chromatograph - time that could not be afforded by the generalist, and so the specialist was born. Now the technology has become so complex - a GC now costs tens of thousands of pounds - and one also has to know how to drive its dedicated computer.

The 1960s were mainly used in development work. The pharmaceutical explosion occurred and the new agricultural chemicals came onto the scene. Thin layer chromatography and gas chromatography were extensively exploited and data collections of ultra-violet and infra-red grew larger and larger. Micro techniques were used and it became rarer and rarer for identification not to be achieved. Credit goes to the person who discovered that the Dragendorf or iodoplatinate general alkaloid revealing reagents formed reversible complexes when used on paper chromatograms, so enabling the original material to be recovered for further analyses. I don't know who it was but it was certainly passed to me by word of mouth. Professor George Clarke was developing his work on microcrystalline tests for alkaloids which eventually led to his magnus opus on the Isolation and Identification of Drugs, of which a new edition is being published this year, edited by Tony Moffat.

The '60s also saw the beginning of toxicology on a routine basis in sport and in the investigation of drugs in accidents involving transportation. Drug abuse reared its ugly head - it was virtually unknown ten years previously in Europe. Cannabis became a growth industry in more ways than one and the criminal fraternity were not slow to move into the hard drug market. Toxicologists began to discover such esoteric facts that morphine may be detectable in bile but not in the brain in the same viscera. The use of metabolites in the investigative process became increasingly important and in cases which previously had been easy - for example a gram of strychnine in the stomach contents, gave way to considerations of "when was the drug taken?" and "is there synergism between these two drugs?". Only now are facts replacing previous guesses which were based on one or two isolated cases.

In relation to metals, the use of neutron activation analyses and atomic absorption spectroscopy came into the field. Neutron activation was a luxury denied to all but a few. Its use tended to be mainly in the field of forensic chemistry - comparison of trace elements in hair being a typical example. I was interested to read a late 1982 paper in a medical journal suggesting that hair was the body material of choice to study errors in metabolism involving trace elements. The argument was that urine reflected what had happened in the body and blood was merely carrying elements from one site to another. As one who was involved

in the forensic chemical analyses of hair, using flameless atomic absorption I counsel caution - millimetre comparisons along the length of individual hairs showed no correlation between hairs plucked from the same head at the same time. Neutron activation analysis for metals is not an expensive panacea - it will not detect lead for example - but my beard is a reminder of when I swallowed arsenic trioxide and followed its excretion in hair using NAA. The experimental work on arsenic in single hairs, nail clippings and sweat is an example of the extreme sensitivity of the method when used with particular metals.

The use of atomic absorption with all its variations made a dramatic impact in the early '70s and I well remember the first time I used it in a case of thallium poisoning. The digestion of large amounts of liver with nitric/sulphuric acid followed by precipitation of the iodide - a procedure which could take days, was replaced by a method which could take direct aspirate of urine and 4, 2g liver samples before noon. The development of special attachments for such metals as arsenic and mercury provided even more potent new weapons in the toxicologist's armoury. It, however, carried with it a major disadvantage - one had to choose the lamps, or later the multi-element lamps, for the metals in which one was interested. Again, no universal search for all toxic metals was easily possible. I attempted by means of a Government contract with a University to get a rotating source of 12 different lamps made but it was not successful. However, the technique was, and is, invaluable. In my hands it enabled the measurement of "normal" cadmium levels in kidney and, as a very quick and easy method of measuring iron in decomposed blood, helped in measuring haem in some work on carbon monoxide levels in relation to putrefaction.

A recent innovation has been the use of X-Ray fluorescence which has a great advantage in that it can detect and measure a wide range of elements. Although it is still largely experimental in relation to detection limits in tissue samples, I do know of a few cases in which it has been successful.

The 1970s saw two new chemical techniques introduced into routine work - HPLC and mass spectrometry. These techniques, in their separate ways, did two things to forensic toxicology. Firstly, HPLC and capillary GC increased the resolution and so purification of non volatiles and volatiles respectively and linked with immunological methods and MS they increased indentification sensitivities into the nanogram region. The separative capabilities were extensively used, and still are, for illicit drug intelligence and the detection and identification of metabolites in cases involving therapeutic pharmaceutical agents. The detection of minor impurities in illicit drug samples enabled the laboratory to relate different seizures, often internationally and in many cases, from the impurities,

to deduce the synthetic route of production and so enabled investigators to look at the supply of precursors.

Again I stress that the number of specialists required for a routine search of poisons is increasing exponentially.

I now turn to another major impact in the 1970s. I group several techniques together under the term "biological". The use of animals to detect poisons has been used since time immemorial - even human animals were used as food tasters by the Roman Emperors - I wonder what happens nowadays! Over nearly 100 years ago textbooks were describing techniques for digitalis detection on the isolated frog's heart and to my own knowledge it was certainly in use in the 1950s. (Then we began to use chromatography and uv). Similarly injections, usually into mice, were widely used and were of inestimable value in the first detected murder by insulin with which I was involved. I remember learning to cut off rats' heads and excising the diaphragm to study its uptake of glucose which is affected by the presence of insulin. The first time this was quite an experience!

However, in the mid '70s the biologists really began to go to town with the antigen-antibody immunological methods using many different detection methods such as radioactivity (RIA) and enzymes (EMIT). Routine measurement of digoxin, insulin, cannabis and many narcotics is now with us - again with the corresponding increases in staff and cost. In the United Kingdom we started by providing an RIA service at Aldermaston in the Central Research Establishment but within a few years the proving and quality control phases were over and regional laboratories entered into the routine work. The detection of many previously unsuspected poisonings has led to the immunological methods being an essential part of routine forensic toxicology. The mind turns itself over when one considers the very small quantity of poison that can be detected - for example in the case of digoxin it is of the order of a nanogram per ml in blood. A current case in Canada involving poisoned babies typifies as an example of what can be done now that only thirty years ago would have been impossible.

Such biological tests have an essential place now in the toxicological laboratory and probably an increasing one as the use of toxins by spy agencies, for example putting poison ingeniously embedded in metal balls fired into the victim, from an umbrella gun, require the toxicologist to be more advanced in his search for "all" poisons than he is at present. To look further into the future the 1980s will undoubtedly see the search for universal sensitivity and selectivity