

MEETING REPORT

MRC/IEH Workshop on The Use of Biomarkers in Environmental Exposure Assessment, Leicester, UK 30 November-1 December 1995

The Institute of Environment and Health (Medical Research Council) and the Department of Environment sponsored a 2 day workshop on the use of biomarkers in environmental exposure assessment. Around 40 participants from academic institutions, research organizations and government attended. The purpose was to review the role that biomarkers can play in assessing total exposure to chemicals by multiple routes and in determining the relative importance of different environmental routes of exposure to the total body burden. The full proceedings will be published by the Institute of Environment and Health.

The workshop was opened by the chairman, Dr D. Coggon (MRC Environmental Epidemiology Unit, UK), who explained that the purpose of the workshop was to consider primarily biomarkers of chemical exposure with the intention of producing a report and identifying the most useful directions for research. Applications for such biomarkers might include epidemiological studies relating exposure to disease outcome, studies to determine the most important sources of exposure, and as a check on the need for environmental controls. The value of a biomarker for a particular application would depend in part on whether it reflected short or long term exposure. There then followed a session comprised of general talks starting with a discussion of the biomonitoring of metals. Dr T. Delves (SAT Trace Elements Unit, UK) revealed that inductively coupled plasma mass spectrometry (ICPMS) is able to determine trace amounts of stable elements accurately (Pt, Mn, Hg, Pb) in microlitre samples of body fluids or tissues, such as blood or preferably urine. The technique is also able to determine the isotopic ratios of elements such as lead. This may allow the source of the exposure to be determined.

Dr P. Farmer (MRC Toxicology Unit, UK) then briefly discussed the determination of DNA and protein adducts in blood cells and DNA repair products in urine. He contrasted chemical specific techniques such as immunoassays or mass spectrometry with non-specific techniques such as ³²P-postlabelling and commented that there may be a 100-fold variation in adduct levels in the human population.

Dr K. Wilson (Health and Safety Executive, UK) talked about solvents and gases in which he highlighted the uncertainty of kinetics at low doses, especially with different routes or patterns of exposure. He mentioned breath analysis using respiratory mass spectrometry for determination of biomarkers of exposure which may be possible some time after exposure has occurred.

Dr A. Brouwer (Wageningen Agricultural University, The Netherlands) discussed biomarkers for dioxins and PCBs. He stressed the important point that understanding the

mechanism of action will often help in devising biomarkers. He explained how this had been done utilizing the Ah receptor reporter gene constructs transfected into cell lines. One example is the chemically activated luciferase gene expression (CALUX) system. Binding of a suitable substrate (e.g. dioxin) to the receptor produces luciferase which can be utilized in an assay system with a sensitivity of around 10 femtomoles. This system shows good correlation with gc-ms assays for dioxin-like compounds. Mixtures of similar compounds can therefore be evaluated which might otherwise be difficult using biomarkers of exposure. This could be useful because the proportions of constituents of a mixture to which an organism is exposed would not necessarily be reflected in the proportions inside the body as different constituents are more or less biologically stable and so have different half-lives. The stable compounds are persistent and often more toxic.

Following on in the same vein Mr H. Mason (Health and Safety Executive, UK) defined and then discussed biological effect markers and susceptibility markers. He developed the idea that effect markers, especially in the occupational health setting where exposures are relatively high, may be more useful than exposure markers. Susceptibility markers on the other hand may raise ethical questions. He then gave some examples of biomarkers of effect and susceptibility concentrating on non-genotoxic areas.

Starting with biomarkers of effect, Mr Mason described how much of the published work had used this type of marker as an indicator of clinical disease. However effect markers may also be used as indicators of exposure. There are a number of reasons for this including the fact that such biomarkers may give information about the dose at the target tissue and can be used effectively to study mixtures. Biomarkers of effect however may be usefully combined with markers of exposure. He then described the biomarkers of effect used in a number of examples including lead, mercury, pesticides and solvents. Mr Mason then talked about biomarkers of susceptibility such as cytochrome P450 isozymes, glutathione transferase isozymes and *N*-acetyltransferase genotypes.

Dr D. Osborn (Institute of Terrestrial Ecology, UK) then raised the possibility of using responses in wildlife as surrogates for humans, as many biochemical pathways are common to both. In some cases the increased susceptibility of wildlife may be useful as an early warning system. Thus general markers in wildlife could be used as tools, a theme which was to be revisited later in the workshop. However there were problems of differences in exposure pattern and extent.

There then followed a series of special sessions on biomarkers in relation to chemicals in air, water and food and finally multiple pathways of exposure. The first comprised a number of short presentations on pollutants in the air and was chaired by Professor J. McMichael (London School of Hygiene and Tropical Medicine, UK). This included metals (Dr D. Gompertz, Institute of Environmental Health, UK), dioxins, (Dr Brouwer, Wageningen Agricultural University, The Netherlands), nitrogen oxides (Dr D. Shuker, MRC Toxicology Unit, UK), benzene/butadiene (Dr P. Farmer, MRC Toxicology

Unit, UK) PM10 particles (Dr R. Maynard, Department of Health, UK), polycyclic hydrocarbons (Dr D. Phillips, Haddow Laboratories, UK).

Dr Gompertz talked about sources of various metals (transport, incinerators and industry) and the biomarkers of exposure available for them. However he pointed out that most is known about occupational rather than environmental exposure. Dr Brouwer talked about sources of dioxins, some of which were the same as those for metals but also included pesticides. These compounds could enter humans in the food chain via air as well as other routes. Biomarkers of exposure included measurement of the parent compound by gc/ms and biomarkers of response included the CALUX system already described. Dr Shuker talked about NO_x and indicated that there are no definitive biomarkers of exposure partly because of endogenous sources. Dr Farmer indicated that exposure to benzene may be via food and water as well as air. The metabolism involves several complex pathways and the mechanisms of toxicity are not well understood. A number of biomarkers of exposure are available ranging from measurement of benzene itself to macromolecular adducts, although there are limitations to a number of these. He explained that 1,3-butadiene is also metabolized by more than one pathway and there are species differences in these pathways. Therefore it is important to measure the biologically effective dose in humans. A number of biomarkers exist including protein and DNA adducts but levels of these are low even with occupational exposure. Dr Maynard then discussed the problem of PM10 particles which may be responsible for a large number of deaths in the UK but for which there are no biomarkers of exposure or effect. Finally Dr Phillips talked about environmental exposure to polycyclic aromatic hydrocarbons (PAHs) for which smoking is an important confounding factor. Specific biomarkers of exposure are required, but even though cotinine (plasma, saliva or urine) is considered the best for ETS, there are dietary sources of this and subject variability.

After lunch the special sessions continued with a series of short presentations on water chaired by Professor P. Calow (University of Sheffield, UK). These included discussion of drinking water (Mr J. Fawell, Water Research Centre, UK), biomarkers in natural waters (Dr P. Matthiesson, MAFF Fisheries Laboratories, UK), metals (Professor M. Depledge, Plymouth Environmental Research Centre, UK), organics (Professor M. Moore, Plymouth Marine Laboratory, UK) and genetic variability (Dr V. Forbes, Roskilde University, Denmark).

Mr Fawell explained that more than a single biomarker of exposure was required if a mixture of pollutants was involved. He divided the situations in which biomarkers are required into two, namely long term exposure to low levels of water contaminants and short term exposure to high concentrations of pollutants resulting from a chemical contamination incident. He said that there was a need for biomarkers of both exposure and effect and that for long term exposure the biomarker needs to give an integration over time. This difficulty may be overcome by using biomarkers which utilize the biologically effective dose such as relatively long lived

macromolecular adducts. However these need to be substance specific. There is a particular need for biomarkers of exposure to chlorination products of disinfection for cancer epidemiology. The ideal biomarker should be non-invasive, reproducible, simple and cheap.

Dr Matthiesson reviewed some of the biomarkers of exposure/effect available for aquatic organisms, pointing out that not all were specific for a particular substance or effect. He also said that in many cases dose response data was poor for these biomarkers. For some compounds, such as surfactants there were no useful biomarkers.

Professor Depledge discussed biomarkers in relation to trace metals. Thus there are many ways in which to analyse metals in tissues (atomic absorption spectrometry, inductively coupled plasma emission, inductively coupled mass spectrometry, energy dispersive X-ray fluorescence and neutron activation analysis for example). However these analyses do not reveal the biological effects of the metals. He felt that more useful biomarkers were those which were integrated indicators of exposure and response, useful for evaluation of mixtures of compounds. He suggested a combined hierarchical approach utilizing a variety of different markers such as metallothioneins, glutathione transferase and reductase, stress proteins, respiratory pigments and physiological effects. Novel potential markers may emerge from techniques such as differential display mRNA analysis evaluating gene activation and suppression, flow cytometry for detection of DNA damage and metal induced resistance.

Professor Moore discussed organic pollutants of aquatic environments and stressed that early warning was essential and that reliance on traditional chemical analysis was not sufficient. In order to interpret the environmental risk, biomarkers which include biological response are needed. A number of these now exist such as cytochrome P450, ras-oncoproteins, vitellogenin, acetylcholinesterase, lysosomal accumulation and DNA adducts. Although most of these are not specific for a particular compound or class of compound, in the environmental situation where organisms are exposed to a multitude of different compounds, biomarkers of exposure/response are preferably not specific. Further research is required to improve understanding of xenobiotic disposition in aquatic organisms and the seasonal and species variations in these, and how the adverse effects relate to the biomarkers.

The session closed with a presentation by Dr Forbes on genetic variability in wildlife. She explained there may be genetic variability in the responses to chemical exposure. For example cytochrome P450 enzyme activities can vary more between strains than because of exposure to xenobiotics. Therefore selection of cell or species specific markers may be necessary. Exposure to the chemical may itself increase variability in biomarker responses. This may decrease the signal-to-noise ratio of the biomarker and hence reduce the detection of potentially hazardous exposures.

A special session on biomarkers in relation to exposure to food pollutants chaired by Dr S. Bingham (MRC Dunn Clinical Nutrition Centre, UK) then followed.

Professor D. Forman (University of Leeds, UK) discussed

nitrate in which he pointed out that although there are biomarkers of exposure such as nitrate in urine, nitrite in saliva, nitrosoproline in urine and nitrosotyrosine in blood, these are confounded by the presence of endogenous NO.

Professor R. C. Garner (University of York, UK) discussed the content of polycyclic aromatic hydrocarbons (PAHs) in food, what risk this posed, how that might be estimated and what role biomarkers of exposure (such as postlabelling and fluorescence assays) might play. He also mentioned food mutagens such as MeIQx and explained how adducts in human tissue could be detected using the technique of accelerator mass spectrometry.

Dr H. Crews (CSL Food Science Laboratory, UK) discussed metals in food for which there were sensitive techniques available as biomarkers of exposure (especially ICPMS). Also a number of biomarkers of response were available such as effects on haem synthesis or stress proteins.

Dr H. Thompson (CSL MAFF, UK) talked about pesticide exposure via food and pointed out that the biomarkers used were 'borrowed' from those used in high level occupational exposure. These included metabolites in urine or adducts to protein, or biomarkers of response such as cholinesterase inhibition. Some of these markers were not necessarily appropriate for dietary exposure which would normally be long term and at a low level creating problems of sensitivity. Exposure to more than one pesticide may occur and some pesticides give rise to multiple metabolites, leading to problems of interpretation. Macromolecular adducts such as from chlormequat and urinary alkylphosphates from organophosphates were however promising biomarkers.

Dr D. Peakall (Wimbledon, UK) talked about organochlorine compounds which have been detected in fish such as in the Great Lakes. However useful biomarkers of effect are not readily available.

The final special session was concerned with multiple pathways of exposure. The chairman, Professor P. Toniolo (New York University, USA) posed the question—'is it possible to discriminate between sources of pollutants using biomarkers, or between routes of exposure and time of exposure (e.g. recent or past)?' Therefore he proposed it would be helpful to use biomarkers in sequence or combination.

Dr N. King (Buckinghamshire, UK) talked about the multiple exposure pathways for lead and pointed out that the particle size is important in relation to amount absorbed and that the best described relationship is between blood lead and that in drinking water; the means of detecting lead exposure and discriminating between sources had already been described by Dr Delves.

Dr Shuker revisited nitrate and explained that by using ^{15}N labelled nitrate it was possible to determine the source, i.e. exogenous or endogenous. The questions were what are the important pathways to measure and are low level exposures even detectable.

Dr Coggon mentioned the sources of exposure to benzene (smoking, petrol, car exhaust, industrial sources) the exposure routes (inhalation, ingestion and skin contact); and the need for biomarkers to identify determinants of personal dose and its interpersonal variation and for use as a measure of

exposure in epidemiological studies such as in relation to leukaemia. Biomarkers available included the compound itself and the metabolites (phenol, trans, trans-muconic acid and phenylmercapturic acid). Unfortunately these biomarkers only reflect recent exposure and are not very useful for long term exposure. Modified histone proteins in bone marrow were suggested as a possible longer term biomarker.

Professor J. Caldwell (University of London, UK) discussed the portals of entry for xenobiotics into the body and therefore the effect route of exposure may have on the metabolic profile of a compound. He also mentioned variability in exposure between and within subjects stressing the fact that the metabolic capacity of an individual was a combination of the genotype and environmental influences such as diet. Thus induction or inhibition of metabolism may occur as a result of combined or previous exposure to other xenobiotics and this may be an important factor. However the overall effect of such factors may be much less than the sum of the component factors *in vivo* because of the interrelationships between them. Therefore the variation in metabolism and bioavailability in different tissues will affect the internal dose or exposure level in that tissue.

Professor Garner briefly described the accelerator mass spectrometer which detects radiolabelled compounds such as ^{14}C . It can for example measure the $^{14}\text{C}/^{13}\text{C}$ ratio but at very low levels of radioactivity. Therefore it is an extremely sensitive means of detecting very low levels of DNA adducts such as might result from environmental exposure.

Mr C. Livesy (Central Veterinary Laboratories, UK) then talked about domestic animals as surrogates for humans in relation to exposure to environmental chemicals in the home. This approach seemed to raise a number of problems however. The Chairperson of each session then summarized what had been discussed and in particular what knowledge was lacking.

The workshop was rounded off by a lecture from Dr P. Schulte (NIOSH, USA) on Biological Markers in Epidemiological Research: Methodological and Ethical Issues. This was a stimulating lecture despite problems with the projector. Dr Schulte described biomarkers of exposure and effect and showed how these overlap and fit into the overall exposure–disease process. He explained that biomarkers improve our understanding of this process and that biomarkers of effect should be early indicators of disease. However whether there will be particular fingerprints identifiable for particular diseases remains to be seen. He indicated that biomarkers of susceptibility impinge at various points in the exposure–disease equation. He then proceeded to discuss biomarkers of each type and some examples in relation to human exposure. Thus biomarkers of exposure to polycyclic aromatic hydrocarbons (PAHs) can be combined with biomarkers of susceptibility such as the activity of cytochrome P450 2D6. This combined approach revealed that the odds ratio for cancer was greatly increased for extensive metabolizers which are also exposed to PAHs. He talked about the need for early markers of effect and the fact that biomarkers should be derived from the critical events in the exposure–disease equation. However the proper use of biomarkers may well require an understanding of the kinetics

of the internal and external exposure. He highlighted a misconception that direct access to biological data means that one is closer to the truth. He then proceeded to explore some of the ethical problems inherent in the use of biomarkers. For example biomarkers should not be used before they are validated and that any uncertainty should be communicated to the subjects involved as well as the results. The question of who owns specimens and whether they can be used for purposes other than those for which they were originally taken must also be addressed.

In conclusion it was clear that although for some xenobiotics (e.g. metals) there are good biomarkers currently available there are many others (organic compounds, particulates) for which there are none. Biomarkers of long term exposure and for exposure to low levels are particularly difficult to find. Markers which give an integrated measure of exposure, which indicate that an interaction with a biological system has occurred and those based on a mechanistic understanding of the toxicity or biological effect are clearly

preferable. It was also clear that there are differences in definition and understanding of biomarkers of exposure and response and the relationship between them. This may be partly due to differences in requirements. For example biomarkers may be particularly useful for validation of other means of exposure assessment such as ambient air monitoring or for use in risk assessment. However the type of biomarker used may well be different in each case. There is also overlap between biomarkers of exposure (e.g. metabolites) and of response (e.g. interaction with the dioxin receptor) where the latter is used as an indicator of level of exposure.

Another relevant meeting is to be held in Lyon on Tuesday 20th February entitled 'Methodological Issues in the use of biomarkers in cancer epidemiology' and the proceedings published by IARC.

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