Direct Indicators of Exposure: Monitoring Chemicals in Tissues, Body Fluids, Urine and Exhaled Air

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1 INTRODUCTION

This paper discusses biological monitoring (BM) for assessment of exposure of ambulant members of the general public.

In recent years, various approaches to assess exposure and/or biological effects have been developed. This has created confusion, because several authors classify all these approaches under BM. Discussion of the definitions is thus appropriate. Because measurement of metabolites as indicators of exposure is reviewed by another contributor, this aspect of BM will not be reviewed. Tissue banking will be discussed only briefly.

2 DEFINITIONS

In 1980, the CEC, NIOSH, and OSHA organised a seminar on "Assessment of Toxic Agents at the Workplace" (Berlin *et al.*, 1984). The seminar agreed upon the following definition of "biological monitoring" (BM):

> Biological Monitoring is "the measurement and assessment of (workplace) agents or their metabolites either in tissues, secreta, excreta, or any combination of these to evaluate exposure and health risk compared to an appropriate reference."

This definition also applies to BM of the general population. Moreover, BM is increasingly applied in clinical medicine (in clinical pharmacology to measure intended levels, and in clinical toxicology to measure non-intended levels) and in animal experimentation. The basic approach can also be applied in assessment of exposure of, e.g., fish, cattle, pets, even plants and ecosystems. This paper adheres to the classical definition of BM presented above.

The seminar defined "monitoring" (as in preventive health care) as "a systematic continuous or repetitive health-related activity, designed to lead

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if necessary to corrective action." Although BM usually is not applied continuously and not always repetitively, repeated measurements to assess trends in time increase the validity of assessment of exposure and health risks.

The definition of BM runs parallel to that of environmental monitoring (EM): both assess exposure and health risk. EM assesses the intake, BM the uptake. They ultimately serve to estimate the dose to the effector organ(s).

BM is based exclusively on the impact of "man on agent," i.e., on toxicokinetic parameters, in contrast to effect monitoring which reflects the impact of "agent on man." The Netherlands recently introduced a definition for monitoring of biological effects (BEM). BEM is

> "the measurement and assessment of early biological effects, of which the relationship to health impairment has not yet been established, in exposed workers (subjects) to evaluate exposure and/or health risk compared to an appropriate reference."

When monitored effects have been established *a priori* as "adverse," then the term "health surveillance" (HS) is used. BEM and HS refer to toxicodynamic parameters, which usually are agent-non-specific, may be contrasted to EM and BM which are agent specific.

Confusion may exist between BM and EM. Measurement of mainly lipophilic agents in breast milk is a BM-method, which, however, is applied primarily as EM. Exogenously deposited metals may become bound to SH-groups; usually it is not possible to make sharp distinctions between exogenously and endogenously deposited metals (Hopps, 1977). Relationships between metal in hair levels and levels in the effector organs are rarely observed (Bos, 1984; Aalbers, 1984) for Cd, Cu, Mg, Mn, Pb, or Zn. When metals are ingested, however, as in the case of methylmercury, BM of hair can be used as an index of exposure. Similar confusion may arise when measuring agents on the skin, in mouth-lavage, or in nails. In practice, such BM-methods generally reflect external dose.

3 ADVANTAGES OF BM

The advantages of BM have been advocated many times (Zielhuis, 1978; IPCS, 1983; Aitio *et al.*, 1984; Berlin *et al.*, 1979, 1984).

BM takes into account the following characteristics:

(1) Total exposure by various routes, from various sources, and from various environments (ambient, occupational, hobby work, nutrition, etc.);

(2) Contributions of physical activity (respiratory volume);

(3) Personal hygiene and personal work habits;

(4) Lifestyle (e.g., consumption of contaminated tobacco, alcoholic and

other beverages—e.g., mineral water may contain substantial levels of arsenic, wine containing lead);

(5) Body stature (female subjects have on average twice the free fatty mass of male subjects), which may affect the level of lipophilic agents in blood and exhaled air;

(6) Individual or group differences in toxicokinetics, e.g., respiratory pattern; respiratory, intestinal and renal absorption and/or elimination; nutrition; differences in biotransformation and in binding to ligands; e.g., in a series of experiments in volunteers with exposure to trichloroethylene one subject always had the highest level of trichloroacetic acid in blood and the lowest in urine (Monster, 1978);

(7) BM may be more cost-effective than EM;

(8) BM levels fluctuate less than EM levels; and

(9) BM offers a more valid indicator of the actual body burden and the dose to the effector organ than does EM.

In both pharmacology and toxicology, the dose should be measured as exactly as possible; i.e., the amount administered and the dose-rate (i.e., the dose per unit of time), and the frequency and duration of administration. In toxicology the unintended dose (rate) can only be approximated, therefore, rationalising the term "exposure."

Various indicators of exposure may be linked to systemic agents (Zielhuis, 1978; IPCS, 1983):

(1) External exposure in a general sense (EM): the concentration in inhaled air, food, beverages, and the frequency and duration of exposure (T).

(2) External exposure in a narrow sense = intake EM. This also requires measurement of the amount of air inhaled, of food ingested, of substance on the skin, taking into account again time. The actual intake may also depend on particle size and water/fat solubility. It differs considerably from that calculated from the concentrations in air, water, or food.

(3) Internal exposure = uptake BM, or the intake × the fractional absorption.

(4) Dose to the target organs (BM).

At least in principle, BM permits a valid assessment of exposure to target organs and consequently better reflects health risks than does EM.

4 LIMITATIONS OF BM

BM also has several limitations, which have been reduced, in recent years, by research efforts.

4.1 NUMBER OF AGENTS TO BE MONITORED

The number of agents that can be monitored by BM is still much smaller than that by EM. In recent years, however, chemical analytical methods have expanded through the introduction of non-invasive physical methods and as a result of increasing insight in the toxicokinetics of numerous substances. A recent review of BM of industrial agents published by Lauwerys (1983a) presented data on 70 agents, many of which are in wide use. In the USA, Pellizari *et al.* (1982) examined breast milk of 48 non-occupationally exposed women living in 4 urban areas. With thermal desorption techniques (GC/MS), they indicated the presence of 172 compounds, albeit at very low levels.

In 1977 a CEC/EPA/WHO workshop on "The Use of Biological Specimens for the Assessment of Human Exposure to Environmental Pollutants" listed 20 agents for which BM-methods were available to assess exposure to the general population (Berlin *et al.*, 1979). In 1983, WHO invited the Coronel Laboratory to update the 1977 report, and to prepare a working paper for the Human Exposure Assessment Location (HEAL) project (1984). Fifty-three groups of agents were discussed; i.e., 33 more than in 1977. Moreover, for each group of agents, the feasibility of using various biological specimens had been more fully established since 1977. We categorised biological specimen into "most feasible" (MF) for BM of the general population; quantitative guides for levels in non-exposed and in exposed subjects are known; "probably useful" (PU), i.e., further research still needed to develop a method for routine use; "not useful" (NU), i.e., the specimen can probably not be used in practice. The HEAL document presents short reviews of the state of the art, and reports relevant quantitative data. In this update, the number of groups of agents was smaller than that for industrial agents; this reflects lower exposure levels in the environment than in the workplace and the wider application of BM in the latter area.

Recently various non-invasive physical methods have been introduced; however, most of them are not available for routine field use. Lauwerys (1983b) reviewed methods to assess exposure to toxic metals. For assessment of exposure to lead in bone and teeth, an X-ray fluorescence technique using a ^{57}Co or a ^{109}Cd gamma-ray source (lower detection limit) for stimulating X-ray production and for lead in teeth and particle excitation and micro-autoradiography have been applied. To assess exposure to cadmium, a portable neutron activation system has been used in several field studies to measure Cd levels in liver and kidneys. Also X-ray fluorescence has been applied, but the detection limit still appeared to be rather high. To assess exposure to metallic mercury, the X-ray fluorescence techniques (^{57}Co or ^{109}Cd) have been used to measure the Hg concentration *in situ* in the superficial tissues of the head. Finnish research workers developed methods to measure iron in the lung in workers in the metal

industry. Chettle (1984) also discussed possibilities for assessment of exposure to strontium, platinum, silicon, copper, and silver.

We may conclude that, although BM still can be applied for much fewer agents than EM, there has been a rapid expansion of the number of agents, many of which occur widely in the environment. Moreover, the quality of BM-programmes has also improved considerably in recent years. There is reason to expect that this expansion of knowledge will continue.

4.2 NOT FEASIBLE FOR SURFACE-ACTING AGENTS?

BM applies primarily to systemic toxicants. However, in exposure of the mucosae to some irritants, absorption also may take place. For exposure to formaldehyde, the compound itself has been measured in blood (Piotrowsky, 1977) and its metabolites in urine (Einbrodt *et al.,* 1976). Gunnison and Palmes (1974) measured the metabolite S-sulfonate in plasma after exposure to sulfur dioxide. Therefore, there is reason also to conduct studies of the feasibility of BM-methods on exposures to surface-acting agents, although the indicator value of the dose to the target organ may be less than for systemic agents.

4.3 INCONVENIENCE

Invasive methods are inconvenient, particularly the sampling of blood, although the introduction of flameless atomic absorption spectrometry (AAS) for measuring levels of metals requires much smaller samples than the old AAS methods. This development made it possible also to sample children 1 to 3 year of age. Sampling blood has proven feasible for studies of large population groups in the USA, in the CEC, and even worldwide.

In recent years, particularly in Europe, sampling of exhaled air has been conducted. For many volatile agents, this measure can replace sampling blood. It has been shown that, in 4 to 5 year old kindergarten children and subjects about 80 years of age, exhaled alveolar air can be sampled with little inconvenience (Monster and Smolders, 1984). One may be able to sample exhaled air when the subjects are at home, after instruction on how to use the vacuum collection tubes. The introduction of non-invasive physical measurement techniques will also increase convenience.

Often authorities choose to sample urine or hair because of relatively easy access. However, BM of hair generally does not permit estimation of internal dose, whereas proper monitoring of urine is easily hampered by dilution and contamination. One should choose a biological specimen that permits the most valid indication of total exposure and health risk.

The introduction of BM-programmes may require much more effort than EM at informing people subjected to this monitoring, to assure that each individual's data be kept confidential. Moreover, it should be made clear

that BM data do not provide evidence on the state of health, but only characterise the actual dose, which in turn may be used to estimate health risks. This should be made clear, because clients tend to treat the personal BM-data similarly to data from clinical examinations in the framework of clinical medicine.

4.4 FEASIBILITY TO ASSESS PEAK EXPOSURES

External exposure may be highly variable over time, and some agents may cause injury as a result of acute overexposure. BM usually is not able to measure peak exposures, a situation which is perhaps more important in the workplace than in the general environment.

5 DEVELOPMENTS IN ANALYTICAL CHEMISTRY

Developments in analytical chemistry are essential for expansion of BM. Four aspects particularly deserve a brief discussion: analytical speciation, increasing sophistication of analytical methods, increased sensitivity of analytical methods, and quality control.

5.1 ANALYTICAL SPECIATION

BM for exposure to metals largely relies on determination of metals in blood and urine. However, compounds of the same metal may differ in toxico kinetics and toxico-dynamics. This applies for instance to arsenic (Zielhuis and Wibowo, 1984). Water soluble inorganic As compounds are metabolised into monomethylarsonic- acid (MMAA) and subsequently into dimethylarsinic acid (DMAA); oral exposure may cause skin cancer and blackfoot disease. Exposure to relatively water insoluble inorganic compounds carries a risk of lung cancer; these compounds may remain for a long time in the respiratory tract. Organic As compounds from marine food are not metabolised and are excreted without change in the urine; health risks rarely exist. BM of exposure to As relies largely on measurement of total As in urine, generally after one day abstinence from ocean foods. In exposure to Cr and Ni compounds, similar problems are confronted. Although measurement of As, Cr, or Ni in urine or Ni in plasma may also reflect, to some extent, inhalation exposure of slowly soluble compounds, levels in blood or urine cannot be relied upon solely for the assessment of exposure to these compounds, which may induce respiratory cancer.

Theoretically, 209 different isomers of polychlorinated biphenyls exist, each with perhaps different toxicokinetics: hexa- and hepta-PCB's have a longer half-life ($t_{\frac{1}{2}}$) than tetra- and penta-PCB's; consequently, the distribution of PCB's in biological specimens may differ from that in EM-samples; the

$t_{\frac{1}{2}}$ is probably inversely related to the capacity to induce enzyme activity (Kuwabara *et al.*, 1978).

Analytical speciation has been much further developed for EM than for BM, and there is a great need to provide such differentiation in BM.

5.2 MORE SOPHISTICATED ANALYTICAL METHODS

This paper cites merely a few examples of how developments in analytical methods have contributed to BM. Detection levels and sample sizes for electrothermal AAS have decreased considerably. This technique has made it possible to carry out studies in young children. Yusho disease in Japan and Taiwan was thought at first to be due to PCB poisoning. However, when it became possible to measure polychlorodibenzofuran (PDCFs), polychlorinated quaterphenyls (PCQ's), and PC-naphthalenes (PCN's), it was concluded that Yusho was primarily caused by these low level contaminants.

5.3 DECREASING DETECTION LEVELS

Developments in analytical chemistry permit measurements of ever decreasing levels. In our laboratory, for instance, the detection levels of trichloroethene and tetrachloroethene in exhaled air have decreased 10- and 100-fold, respectively. Pellizari *et al.* (1982) using GC/MS found 172 compounds in breast milk at very low levels.

The application of methods with low detection levels depends upon

(1) biotransformation products of minor pathways;

(2) intensity and duration of external exposure. Our laboratory examined non-occupationally exposed subjects living above and next to chemical cleaning shops in the centre of Amsterdam; in workers, the concentration in alveolar air was 10 to 300 mg/m^3; in subjects living above the shops 0.3 to 100 mg/m^3; in those adjacent to the shop $>$ 0.1 to 30 mg/m^3; in those next door (second house) $<$0.1 to 3 mg/m^3; and in controls merely $<$ 0.1 mg/m^3 (detection level) (Verberk and Scheffers, 1980). However, in a later study of kindergarten children near a factory in a rural area and in an old folks' home near a waste dump, we applied a method with a very low detection level; we measured levels of about 6 to 20 μg/m^3 (D.L. = 1 μg/m^3) in alveolar air (Monster and Smolders, 1984).

(3) Past exposure situation. Measurements were made of 10 to 100 μg tetrachloroethene/m^3 alveolar air up to 3 months after occupational exposure; with a half-life of 6 to 8 days. Past exposure could be estimated to have been about 100 mg/m^3.

These examples illustrate the advantage of analytically sensitive methods. However, this is also being criticised, because it may create unwarranted fear. The presence of agents in biological specimens is too easily interpreted

as evidence of health risk. Such findings require extra attention to inform the public. Nevertheless, in establishing internal exposure/response relationships, the entire range of exposures needs to be investigated to establish the no-adverse-response level.

5.4 ANALYTICAL QUALITY CONTROL

In the last decade, there was an increasing emphasis upon quality control. The precision and accuracy of the analytical procedure have to be established. This requires good laboratory practice and inter- and intra-laboratory control procedures. The procedures can be briefly summarised as follows (HEAL, 1984):

(1) use of reference methods;

(2) use on routine basis of control materials;

(3) participation on periodic basis in quality assessment programmes (e.g., round-robin studies); and

(4) assistance of an expert for certain types of measurements.

Routine BM-programmes should be carried out exclusively by laboratories which participate in such quality control programmes, and with methods for which the precision and the accuracy are known.

6 MEASUREMENT STRATEGIES

Often, BM is carried out without a defined strategy or a specific objective. Three general objectives may be possible:

(1) assessment of the existence of overexposure in specified areas or groups of subjects;

(2) assessment of the intensity of exposure; and

(3) to assess exposure in specified individuals, perhaps with an alleged or actual intoxication.

For the last objective, BM may rely on one sensitive parameter; e.g., the lead concentration in blood (PbB) or the Cd concentration in urine (CdU). When the PbB level is below 150 to 200 μg/l, there probably was no overexposure; the same applies to CdU <2 to 3 μg/g creatinine. When the level of trichloroacetic acid in urine is less than 75 mg/g creatinine, then exposure very probably does not exceed 50 ppm for 8 hours/day for 5 days/week. To assess the magnitude of overexposure, one may have to add other parameters of BM or even of BEM. For lead, EDTA-provoked-Pb in urine and zinc protoporphyrin in blood should be included; for cadmium, Cd in blood and retinol binding protein (RBP) in urine should be added. For exposure to chlorinated hydrocarbon solvents, one should rely on levels of the agent in alveolar air or in blood, combined with concentrations of metabolites in blood or urine. Combining several BM parameters may

increase the predictive validity of assessment of exposure (Monster, 1985). In addition, repetitive measurements over time will permit more reliable assessment of exposure. In an individual with alleged or actual poisoning, one may also need to measure clinical parameters, and to look for trends over time.

A valid measurement strategy should begin with a very well defined question; e.g., does one want to assess exposure to agent A on the day of measurement, even over the last hours, or a time-weighted average exposure over the last week, or an accumulated exposure over years? The measurement strategy depends upon the specified objective, the toxicokinetics of the agent, the exposure history (variability, frequency, duration). As an example, in assessing exposure to methylene chloride for 3 to 4 hours, measurement of the agent in alveolar air and/or blood is the method of choice; but for longer exposures, one should measure the level of the metabolite carbon monoxide (CO), in air or of CO or COHb in blood. The $t_{\frac{1}{2}}$ of COHb in blood is even longer than in exposure to CO itself. Confounding exposures to tobacco smoke, alcoholic beverages, pharmaceutical drugs may also have to be taken into account. In exposure to agents with a long $t_{\frac{1}{2}}$, the point of time of sampling is less critical than when $t_{\frac{1}{2}}$ is < 24 hours, when sampled < 60 minutes post exposure. When sampling solvents in the first 30 minutes after exposure, usually $t_{\frac{1}{2}}$ is very short; this often allows one to estimate immediate past exposure.

Before starting a programme, one should be able to answer the six W's (Zielhuis, 1978; IPCS, 1983).

(1) What agents need to be studied?

(2) Where should samples be drawn?

(3) What quality of data is needed?

(4) Which instruments or techniques should be used and which groups of subjects should be examined?

(5) Why: does there exist a biological hypothesis on the exposure/response relationship?

(6) When: sampling time in relation to exposure?

The answers may differ among compounds, groups at risk (*inter alia*, personal habits, age, sex), and availability of budget and expertise.

No BM programme will permit a valid conclusion in a cost-effective way, if one does not follow a well-defined strategy of measurement.

7 INDIVIDUAL BM

The majority of biological guidelines refer to group mean levels; most apply to occupationally exposed subjects. Although one accepts the fact that levels in individual subjects may vary rather widely around this mean, one still hardly knows why they do so. Does this reflect variability in exposure?

hereditary differences in toxicokinetics (e.g., inborn errors in metabolism or capacity to respond with enzyme induction)? acquired differences in elimination from the respiratory tract, in liver or renal function? interaction with drugs? consumption of alcoholic beverages? obesity? physical activity? age? sex?

The ultimate objective of exposure assessment is to establish the dose in the critical organ. At least in principle, BM offers a better approach to assess total individual internal dose than does EM. Combining several BM parameters may already add useful information, but the standard error of the estimate may remain large (Monster, 1985).

Measurement of PbB alone does not fully take into account the variability in the distribution of lead throughout the body, which may vary between individuals and which is age-dependent. For past exposure to lead, EDTA-provoked PbU-levels provide a closer correlation with protoporphyrin levels as indicators of early biological response than with PbB. Moreover, protoporphyrin levels at the same PbB-level are higher in young subjects than in adults, and higher in adult women than in men. Combining parameters of BM with parameters of BEM may increase the validity of assessment of individual health risk.

Toxicokinetic models usually are tailored for a "standard man." By changing the free fatty mass, one can estimate the changes in distribution of volatile lipophilic agents among body compartments.

An interesting new approach has been developed in the Coronel Laboratory by Opdam (Zielhuis, 1984). This approach measures indirectly the impact of individual factors which modify toxicokinetics by establishing the so called Impulse/Response Curve (IRC), i.e., the curve of the concentration of the agent in mixed venous blood over time, when the agent is administered instantaneously. The IRC is obtained by monitoring alveolar air. In human volunteer studies, Opdam showed that the $t_{\frac{1}{2}}$ for tetrachloroethene, measured between 6 and 15 days after exposure, varied among 3 male subjects from 3.7 to 4.8 days, and among three female subjects from 4.3 to 6 days. Moreover, in exposure to trichloroethene, physical activity (60-watt) was shown to alter the biotransformation rate for trichloroethene, but not for tetrachloroethene. The percent of trichloroethene exhaled increased, perhaps as a result of enhanced circulation in body fat and muscles. This was not the case for tetrachloroethene, which undergoes little biotransformation.

These examples illustrate that much research is still needed before one can confidently ascertain internal exposure in individuals and to estimate health risks.

8 BM AND EM: SUPPLEMENTARY OR COMPLEMENTARY TO EM?

For many agents to which humans may be exposed, no BM-programmes are available; EM offers the only approach feasible. In compliance monitoring, one has to apply EM when the standards are expressed in EM parameters. However, when one wants to assess whether overexposure exists and when BM-methods are available, BM may be much more cost-effective; when pre-established biological exposure guidelines are not exceeded, then EM is not needed for establishing the sources of pollution. The CEC would not have been able to assess exposure to lead in the nine member states by means of EM as quickly and with the same budget as was possible by the large scale BM-programme (CEC, 1981). The same is true for the NHANESS-study in the USA and the worldwide WHO-programme (Vahter, 1982). In the skin cancer study in Taiwan, if BM had been applied, the assessment of exposure to water soluble inorganic arsenic would have been achieved reliably and would have increased confidence in estimates of skin cancer risk.

Brunekreef (1984; 1985) recently published a critical review of the relationship between the lead levels in ambient air (PbA) and in children's blood (PbB) in children. For adults, one usually applies a ratio of about 1 to 2 between PbA (in $\mu g/m^3$) and PbB (in $\mu g/100$ ml), assuming that adults are exposed to lead for 24 hours/day, an exaggeration of actual exposure (IPCS, 1977). For children, a somewhat higher ratio may be used, reflecting the fact that the relative contribution from non-respiratory intake is much larger than for adults. In about 20 studies, the ratio PbA/PbB varied from <1 to about 16, at low PbB-levels usually higher than at high PbB's; even when adjusted for non-respiratory intake (excluding intake through food), the ratio still ranges from 1.5 to 8.5. Assessment of external exposure by EM would have required sampling and analysis of various environmental media, of which air as such is relatively unimportant.

From the health point of view, BM should always be preferred to EM, because theoretically BM approximates the dose to the effector organ more reliably than does EM. When internal dose/response relationships (DRR) have been established, then the health risk can be estimated. However, there still exist comparatively few DRR's based upon BM-parameters of exposure; examples are those for lead, cadmium, inorganic metallic mercury, and alkyl-mercury. Most DRR's are based upon assessment of exposure by EM of air, food, and drinking water. The relationship between parameters of EM and of BM usually are secondarily derived from studies designed for this purpose. Consequently, replacement of EM by BM data does not automatically improve the dose/response relationship, particularly when exposure occurs through routes other than that assessed by EM. BM is able

to assess accumulated exposure over a long period of time (long $t_{\frac{1}{2}}$); e.g., by measuring Cd in urine, PCB's in blood, in adipose tissue or breast milk, and DNOC in blood. In such cases, BM-parameters may approximate past exposure better than EM. Where possible, one should carry out both BM and EM in prospective studies.

The CEC/NIOSH/OSHA Seminar (Berlin *et al.*, 1984) mentioned the following reasons to apply EM:

(1) compliance monitoring to EM-standards;

(2) establishment of the relationship between exposure and health effects;

(3) assurance of the effectiveness of control measures;

(4) evaluation of the need for controls in the vicinity of specific sources of emission;

(5) trends in improvements or deteriorations in the workplace or the environment; and

(6) an historical record.

The same reasons can be given for BM, except maybe for reasons (3) and (4) above.

9 BIOLOGICAL GUIDELINES

For occupational health, interim guidelines exist for about 40 widely used agents, some based upon agent-specific parameters of BEM. Most guidelines refer to group mean levels, while a few refer to individual maximum levels such as those for lead, cadmium, DNOC, and CO.

To monitor non-occupationally exposed subjects, the Biological Guideline for lead is an outstanding example. This was based upon a Biological Quality Guide (BQG), proposed by Zielhuis (1974); the BQG refers to the percentile distribution of PbB-levels in representative groups of subjects (n = 50 to 100) in defined areas; the reference distribution was 50 percent <200 μg Pb/l; 90 percent <300 μg/l, and 98 percent <350 μg/l. The number of subjects (adults and/or children) to be examined and the areas to be sampled were defined in the CEC Directive (CEC, 1981). In 1979, about 5800 children, 100 pregnant women, and about 12,000 adults were examined. In 1981, a second programme was carried out. Overall, the distribution was usually below the guideline. When specific sources of emissions existed, then the guideline might be exceeded somewhat, particularly in children. The levels in control urban areas were usually higher than those in rural areas. This large scale study pinpointed the areas where subsequent EM needed to be conducted.

Vahter (1982) assessed human exposure (teachers from different parts of the world) to lead (PbB) and cadmium (CdB) in 10 countries (100 to 200 per country). In 8 countries, the Cd level in the kidney cortex also was

measured (about 30 to 150 per country. In the 60's, Goldwater and Hoover (1967) carried out a study of PbB-levels in 16 countries. For assessment of exposure to chlorinated hydrocarbon pesticides, many studies have been carried out. However, these studies were not designed as compliance monitoring to a preset Biological Guideline as was the case in the two CEC studies, but they showed the possibility of carrying out internationally coordinated BM studies. There is need to agree upon preset percentile distributions for a wide range of compounds.

When one designs a longitudinal study, trends over long periods of time can be assessed. Results from such studies may provide highly compelling arguments for preventive control actions.

10 SPECIMEN BANKING

In the last decade, a new development took place for both EM and BM. In 1977, the CEC/EPA/WHO (Berlin *et al.*, 1979) organised a Workshop on "The use of biological specimen for the assessment of human exposure to environmental pollutants." This workshop not only reviewed various methods of classical BM but also specimen banking, i.e., "the systematic collection and storage of samples for deferred examination. These activities will be deferred for a period of years or even decades following collection." Schmidt-Bleek and Muhs (1979) discussed the conceptual design. The principal reasons for establishing such a specimen bank are:

(1) to provide real-time information on the distribution of man-made chemicals and of decomposition products in the environment;

(2) to examine trends with respect to increasing threats; and

(3) to preserve for a long period aliquots of samples which were originally analysed for future classification of specific problems.

Both in the USA and in the FRG, activities to establish such banks started in the 1970's. The authors contended that each country will eventually have to develop a grid-type determination and display of the state of the ecology and permissible ecological load factors. Rook and Lafleur (1979) mentioned the following categories of specimens:

(1) soft tissues of human origin;

(2) food material, particularly grains;

(3) samples of water-borne organisms, particularly plankton and oysters; and

(4) biological indicators of airborne particles, particularly lichen and moss.

In 1978, the CEC/FRG/EPA organised a second workshop on specimen banking (Luepke, 1979). It established specific criteria for sample selection. Additional objectives included: (1) facilitation of base-line trends; (2) analysis of stored specimens for substances just recognised as pollutants; (3)

increased knowledge of the relationships between chemicals and their toxic effects; (4) retrospective dose-estimates; (5) deferral of analyses until better information becomes available regarding the most appropriate materials to be analysed; (6) application of improved analytical methods; and (7) comparisons of subsequent analytical data with previous data. After a pilot phase in 1976 to 1984 a final system of specimen banking started in the FRG in 1985.

It is too early to discuss the assets of this new approach both in BM and in EM, but the present objectives promise to eventually provide data highly relevant for preventive and corrective action. One wonders if and when it will become possible to start a worldwide activity of specimen banking in close cooperation with classical worldwide BM programmes.

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