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Collection, Processing and Storage of Specimens for

BIOLOGICAL MONITORING OF OCCUPATIONAL EXPOSURE TO TOXIC CHEMICALS

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COLLECTION, PROCESSING AND STORAGE OF SPECIMENS FOR THE BIOLOGICAL MONITORING OF OCCUPATIONAL EXPOSURE TO TOXIC CHEMICALS

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BIOLOGICAL MONITORING OF OCCUPATIONAL EXPOSURE TO TOXIC CHEMICALS.

An Introduction

Biological monitoring of workers exposed to toxic chemicals has gained increasing attention as a means of assessing the hazard to health (4,5,15, 85,93,118,181). Biological monitoring is primarily an activity where repetitive measurement of toxic chemicals in biological specimens is used to assess the exposure levels of individual workers and of groups of workers. More recently attempts have been made to use this kind of information to produce limit levels which could ensure that no relevant ill health would ensue from the exposure. An example of such an effort is the booklet "Recommended health based limits in occupational exposure to heavy metals" (175).

In some instances an effect of the toxic compound on certain physiological or biochemical functions has also been measured as a part of biological monitoring. The current report is limited to measurement of toxic chemicals or their metabolites. Due to developments in analytical chemistry, a wide variety of toxic compounds, at least 100 chemicals or their metabolites may be analysed in human specimens. At present the main obstacle in broadening the application of biological monitoring is not the problem of analysis, but lack of information on toxicology and toxicokinetics of the chemicals in question.

When interpreting results of biological monitoring for risk assessment one should be aware that the accuracy of the result is usually not known and may, especially in the past, have been poor. Striking examples of demonstrating inaccuracy - mostly due to contamination - are recorded in the recent history of the analyses of trace elements.

Very little is known of the relationship between human physiology and pathology or the exposure conditions and the concentrations of the compounds which were found in human specimens. This is especially true of organic chemicals which may be absorbed into human body both by inhalation and through the skin.

Because many toxic compounds are rapidly cleared from blood (excreted or stored eg. in fat), analysis of specimens without standardizing the time of specimen collection is practically worthless.

The analytical results from biological monitoring are interpreted by comparing them with reference values from other populations. In occupational monitoring, there are three hierarchies of reference values.

Reference values for a population without occupational exposure to the chemical. In recent years considerable progress has been made in the production of these values.

For the exposed populations there are in principle two different sets of reference values: For a few chemicals, good information is available on the health effects, even in long term, of certain levels of exposure. Thus one may state with fair confidence that when the values observed do not exceed these health based limits for the exposed, no untoward health effect is to be expected. Such are the reference values for eg lead and mercury. However, for the vast majority of toxic chemicals, no health-based reference

values are available. In this case, the reference values are usually derived from the occupational hygienic standards, as a mathematical approximation to correlation between concentrations in air and eg. in urine. In this case a value within the reference interval does not guarantee absence of untoward health effects any more than the corresponding hygienic limit value; the hygienic measurement may even be better.

Reference values are costly to come by. Therefore, whenever possible, one should try to avoid the situation, in which every laboratory has to work out its own reference values. The only way to achieve this, is to make sure that the measurements that are performed are correct. The measurement process incorporates all the phases from the evaluation of the physiological state of the worker, and specimen collection, to the recording of the result.

The present document briefly reviews sources of error in the procedures of measurement and pitfalls in the interpretation of results of biological monitoring. Analytical errors will not be considered here. Various aspects on sources of error in biological monitoring of exposure to chemicals have been reviewed (1,8,16,57,74,81,134,149,150,168). This document will be complemented by practical guidelines specific of various chemicals. The first of these guidelines is in preparation (E. Nieboer and coworkers).

SPECIMENS IN USE

Urine and blood specimens have been most commonly used for biological monitoring of occupational exposure to toxic chemicals. Some compounds have been measured in exhaled air or adipose tissue (17,23,27,43,44,50,79,179), but little has been done for the standardization of these specimens so that they still belong to research rather than everyday practice.

Keratinised tissues have also been used in the monitoring of exposure to several toxic trace elements (37,61,165). The body burdens of lead and cadmium have been studied by analyzing teeth. (eg. 109)

PHYSIOLOGICAL SOURCES OF VARIATION

Blood specimens

Physiological factors that contribute to the intraindividual variation of blood constituents have been reviewed recently. (146,147,180) Data on the effects of physiological changes on the concentrations of toxic chemicals in blood are scanty, but it seems inevitable that physiological factors should have marked effects also on measurements of toxic compounds or their metabolites.

Distribution of water depends on posture: standing up leads to a loss of plasma water mainly to lower extremities, and thus to an apparent increase of appr. 10% in the concentration of nondiffusible blood constituents like proteins or cells. Posture-dependent changes may be much more marked in disease states. (eg. 40)

Similar changes in water distribution take place locally, when a tourniquet is used for blood specimen collection: Water leaks from blood vessels into the tissues of the forearm (147,180). Most toxic chemicals are transported in the blood bound to proteins or cells. Changes in posture and application of tourniquet will therefore change their apparent concentrations.

Exercise - even of short duration - causes changes in blood serum constituents, possibly due to leakage of intracellular components, eg enzymes from muscles. Exercise that lasts longer may have very profound effects on human clinical chemistry. Continuous training may cause hemodilution; this may lead to apparently too low values of toxic compounds. Circulation and ventilation increase at work. This enhances the uptake of inhaled chemicals, e.g. organic solvents (26,103,127,128,182, 183). The role of physical stress in the uptake kinetics of chemicals from aerosols through inhalation has not been studied. Theoretically, the effect of physical stress on their uptake might be even more marked. Changes caused by physical strain may even be seen in urinary excretion of trace elements, as exemplified by a 5-fold increase of the urinary chromium excretion after 2 h-running (10).

A meal, especially food rich in fat increases the concentration of lipids in serum. Most lipid soluble compounds in the circulation are associated with serum and cellular lipids. The increase in blood lipids after a meal therefore leads to a redistribution of such chemicals between air, blood and other tissues. Thus, the venous blood/alveolar air concentration ratio for dichloromethane was directly related to the content of triglycerides in the blood (117), and that for m-xylene was higher after a meal (124). It is to be remembered also that an increase in serum lipids leads to enhanced turbidity that may intefere with analyses.

During pregnancy marked changes are seen in the water balance and distribution; the increase of plasma volume by a third elicits changes in the concentrations of many blood components. (eg 73,180)

Many pathological conditions may affect blood or serum concentrations of toxic chemicals. The liver is the main site of metabolism of organic chemicals. Several trace elements (eg. lead, arsenic, zinc and copper) are excreted primarily through the liver. Liver disease or cholestasis thus probably decrease their clearance (e.g. 80). On the other hand, kidney damage causes retention of those chemicals (e.g. Al, F), whose main excretory route is through the kidneys (42,97,137,177). Similarly, the kinetics of compounds excreted through the lungs are probably changed due to chronic bronchitis or emphysema (e.g. 25). Errors caused by physiological variation may - and must - be decreased by standardization of specimen collection. This has been thoroughly treated (6).

Urine specimens

As a rule, only 'spot' urine specimens have been available for biological monitoring of occupational exposure to toxic chemicals. Because the excretion of various solutes into urine may vary to a great extent, even in healthy people, (142,180) some standardization for urinary excretion rate has had to be used. The most widely used approaches to standardization have been based on relative density, the concentration of creatinine in urine or simply the length of the urine collection period. Osmolality, which is used in hospitals, has been very little applied in biological exposure monitoring.

Correction using relative density. The relative density is mainly determined by the main solutes in urine. The rationale of the relative density correction is that the amount of solutes excreted remains the same, regardless of the amount of water in which the solutes are dissolved. Excretion of proteins or glucose in urine in disease states may negate this approach to standardization.

Correction using creatinine concentration. Excretion of creatinine has been used as an indicator of the completeness of diurnal urine specimens in clinical laboratory medicine. Normally it is fairly constant in an individual, but it varies to a great extent between individuals and between the sexes. Creatinine is the end product of catabolism of muscle creatine phosphate, and its excretion is best correlated with the muscle mass or total body weight (39). The output of creatinine is little affected by diet or diuresis but it is to some extent by physical stress. Renal function may be impaired considerably before the urinary excretion of creatinine is decreased.

Correction with period of excretion. Collection of timed urine samples is the routine practice in clinical medicine, but in biological monitoring of occupational exposure, the collection of 24-h urine specimens is almost impossible. A short term timed collection could be a more practical approach, but information on the applicability of such a method of standardization is not available.

The value of both creatinine and relative density standardization has been questioned (41,62,121). The published data and opinions vary greatly: Tola et al. (155) found that the best correlation between nickel in air and urine in electroplating workers was achieved when the urinary specimens were standardized by the relative density method. No difference was found between the two methods by Morgan and Rouge (106) in workers exposed to Ni dust in a refinery. Tola et al., on the other hand, had found previously (156) that creatinine correction was better than the relative density correction in improving the correlation between chromium in urine and air in metal arc welders of stainless steel.

The value of these two standardization techniques is further obscured by the diurnal variations in the urinary excretions of some trace elements eg. mercury and manganese (76,119,173). The best method of standardization evidently depends on the handling of the chemical in the kidney, and thus is probably different for different chemicals.

In kidney damage the excretion of both water and the solutes into the urine changes. The urinary excretion of cadmium is increased in cadmium-induced kidney damage. Kidney damage usually decreases the clearances of compounds that are mainly excreted through the tubuli (39,51,120).

Severe stress leads to increased transpiration and to relative water deficiency. When the kidney function is normal the urine excreted is then more concentrated. Data on the changes in the excretion of organic compounds, metals and metalloids in these conditions are lacking. It has been suggested (41,121) that when the urine is very dilute or very concentrated, relative density loses its values as a method of standardization of urinary excretion of solute.

ENVIRONMENTAL SOURCES OF VARIATION

Toxic chemicals in body fluids are not necessarily derived from exposure at work: diet, and free-time activities may constitute important alternative chemical sources. It is evident that the toxicity of a chemical is not dependent on its source; however, estimation of occupational exposure may be completely misleading if these other exposures are not considered. In addition, chemicals and other environmental factors may change the fate of toxic compounds in the organism. This, too, might render the interpretation difficult.

Diet. Diet may be an important source of certain toxic compounds in man: Urinary concentration of arsenic is directly related to arsenic in drinking water (163,176). Sea food contains organic arsenicals that may increase the urinary concentration of arsenic many fold (eg 24,164,176). Dietary chromium is reflected in the urinary chromium (9,11,83,161). The concentration of cadmium in the soil is reflected in the cadmium of food stuffs, and finally in the urine (eg. 54). Fish may contain extremely high concentrations of methyl mercury, which usually becomes included in the analysis of blood mercury used as an indicator of occupational exposure (174). Monitoring exposure to benzene, toluene, or styrene from the urinary concentrations of phenol, and hippuric and mandelic acids, respectively, is severely handicapped because the diet may contain high, and variable amounts of chemicals that are metabolized to these end products (85). Wide-spread environmental contaminants such as PCB's and DDT are detectable in individuals never exposed to them occupationally.

Smoking. Smoking has several effects on biological monitoring. Tobacco smoke contains e.g. lead and cadmium, and smoking seems to double the daily cadmium intake of a smoker compared to a nonsmoker (e.g. 136,162). The carbon monoxide from the combustion of the cigarette binds to hemoglobin in blood. The amount of carboxyhemoglobin thus generated may be larger than that due to occupational exposure to carbon monoxide. Cyanide from tobacco smoking may similarly interfere with biological monitoring of cyanide exposure. (172). Tobacco smoking seems to cause enhanced uptake of lead in workers exposed to lead (e.g. 21), and a similar trend has been observed also with eg. nickel and chromium (77).

Alcohol. People who drink alcohol seem to have higher concentrations of lead in blood than abstainers. The basis of this observation is not known (138). Alcohol has several effects on human physiology that may influence the results of biological monitoring. An acute heavy dose causes hypertriglyceridemia, and slight metabolic acidosis. The increase in triglyseride concentration is accentuated in long term consumption; simultaneously, the cholesterol bound to high density lipoprotein increases. Alcohol may also cause fatty degeneration of the liver, which may impair the flow of bile (56). Alcohol may inhibit the metabolism of other compounds. In man, this was first shown with trichloroethylene (107). Alcohol slows even the metabolism of phenylethane 1,2-diol - metabolite of styrene - to mandelic acid. (178) Riihimäki et al. (126,129) found that alcohol may interfere with the metabolism of xylene in human body. These findings indicate that alcohol may mislead the estimation of exposure

obtained from concentrations in biological specimens.

Concomitant exposures. Several hobby-related exposures may form important sources of exposure. These include exposure to styrene in the building of glass fiber reinforced plastics boats, and exposure to benzene, or mixture of aliphatic and aromatic hydrocarbons, lead and cadmium, in car repair work or exposure to pesticides in gardening.

Changes in metabolism. Several organic compounds have been shown to change the capacity of man to metabolize toxic compounds. These include DDT, PCB's, alcohol and several drugs (see 2). However, the importance of these changes to the clinical laboratory sciences has been recognized only recently (e.g. 58). In experimental conditions also several trace elements have effects on the xenobiotic biotransformation system. Some chemicals e.g. carbon disulphide may abolish certain types of cytochrome P-450, and thus lead to altered metabolism of organic compounds (94,112).

KINETIC SOURCES OF VARIATION

Many chemicals are distributed in several compartments in the body; therefore their disappearance from eg. blood exhibits several consecutive half-times. For a number of organic solvents, the first half-time, corresponding to the compartment comprising the circulating blood is very short, i.e., the concentration of the solvent in blood decreases very rapidly immediately after the cessation of the exposure. For example, the first half-time of the disappearance of toluene from blood is appr. 2 min. (32) This means, on one hand, that by varying the time of the specimen collection one may estimate exposure over different time periods. Thus the concentration of toluene in blood directly after a peak exposure reflects the level of the immediately preceding peak, the specimen taken after working hours reflects the exposure during a few preceeding hours; and the specimen taken 18-20 h after the end of the exposure, the mean exposure over a whole day.

On the other hand, to be able to interpret the varying concentrations under these circumstances, the time since exposure must always be strictly standardized when the specimens are to be collected after the work shift.

One must, however, note that information on the toxicokinetics of various chemicals is still very limited. Also the data for organic solvents mainly come from experiments in exposure chambers where the situation may not be similar to the work place.

Skin absorption. Many organic compounds easily penetrate intact human skin. Such compounds include phenol, chlorinated phenols, several aromatic or chlorinated solvents, amines, organic nitrates, and several pesticides (13,118,135,148). Use of gloves may be ineffective in protecting against skin absorption. (71). When the hands of the worker have been in contact with a chemical absorbed through the skin, its concentration in the venous blood collected from a cubital vein indicates a 'local' rather than total body uptake of the compound. The difference in the concentration between the two hands may be up to fifty-fold (47,70,125). For metals, skin absorption is probably not of importance (eg. 3).

VARIATION ASSOCIATED WITH SPECIMEN COLLECTION AND STORAGE

The range of chemicals analyzed for the purposes of biological monitoring of occupational chemical exposures is wide - from volatile solvents to heavy metals. Therefore, the problems encountered in specimen collection, processing and storage are diverse. The available data on the circumstances in which specimens should be stored is scattered, and for many organic chemicals practically no information is available. The existing information is also often hidden in the published articles. In many cases, important information on specimen collection and storage lack completely in published papers. A definite step forward would be, if scientific journals - not only analytical - would require inclusion of pertinent data in all published papers. Little information is available on long-term stability, as needed for example in the preparation of quality control specimens. The stability of a chemical may be vastly different in different matrices, eg. in distilled water, sea water, or a biological fluid such as urine and blood.

The concentration of the analyte may also affect the stability profoundly.

The factors most likely to result in erroneous analytical results during sample collection and storage include contamination in different phases of the analysis, shifts between compartments of the specimen, exchange with the container material, evaporation of analyte or solvent, chemical deterioration because of metabolism by the biological matrix, or by bacteria, and changes in the matrix that may change the analytical recovery. The relative importance of these processes is different for different chemical classes of analytes. For example, evaporation is a problem with organic solvents or metallic mercury; metabolic changes tend to destroy various organic chemicals, whereas contamination is a common problem in trace element analysis.

Loss of analyte

Evaporation. Organic solvents which are routinely analyzed in biological specimens for assessing occupational exposures include tetrachloroethylene, toluene, 1,1,1-trichloroethane, trichloroethylene, and methylethylketone. Some of them are quite volatile, but no systematic study on how rapidly they are lost from biological samples is available. Curtis and coworkers (36) studied loss of pure solvents from different vials and showed that ether, n-hexane, methyl isobutylketone and kerosene were rapidly lost from polyethylene vials with a screw cap. Remarkable losses of n-hexane were recorded within a year even from polypropylene, high-density polypropylene, and Teflon-FEP containers. Losses were smaller in glass containers, but still between 3 and 12%/year depending on the stopper. Loss of ethanol, methylisobutylketone and kerosene were 0.02 to 3.7% annually in glass bottles, depending again on the type of the stopper.

It is probable that losses from solutions with very low concentrations - such as for toxicological analyses - are much smaller. It is also probable that the greater solubility of many solvents in blood (containing lipids) than in pure water tends to decrease the evaporation losses. Thus it has been reported that standard solutions of benzene, toluene, trichloroethylene and ethylbenzene in bovine blood, stored at 4°C in full glass flasks with ground-glass stoppers remained stable for at least 3 weeks (122). 1,1,1-Trichloroethane in blood from exposed workers was stable at +5°C for a week (Pekari K, unpublished). Nevertheless the stability of specimens for volatile solvent analysis should always be verified. Use of tightly closing containers, filling the vials to the rim, and storage in the cold, should decrease losses by evaporation.

Chemical deterioration. Information about chemical deterioration is also very scattered; it is, however, clear that many organic chemicals have limited life span in biological matrices. The biological matrix may contain enzymes or other catalysts that degrade chemicals; in urine samples, bacteria may be very effective in the biotransformation of a variety of organic molecules.

A loss of 25% of phenylglyoxylic acid, a metabolite used as an indicator of exposure to styrene, was demonstrated in urine after 4 hours at room temperature. (66) Even in the refrigerator, loss of phenylglyoxylic acid was remarkable in 2 weeks. (45) However, mandelic acid, another metabolite of styrene, was stable in urine at -25°C for several months (65), and for 2 weeks even at room temperature. (45)

Pentachlorophenol, a wood preservative, remained stable in urine for a week in the refrigerator, but storage for three weeks even in the freezer was reported to result in a 20% loss. (123). 2,3,4,6-Tetrachlorophenol and 2,4,6-trichlorophenol, on the other hand, in urine samples from exposed workers were stable for at least two weeks at +5°C. (Pekari K, unpublished)

Urine specimens for phenol analysis - in the monitoring of exposure to benzene or phenol - could be stored at 4°C for a week, and for at least a year in the freezer without losses. (123)

2-Thiothiazolidine-4-carboxylic acid, used for the monitoring of exposure to carbon disulphide (133,166), was stable in urine for 40 h at room temperature, at 4°C and at -20°C, but showed a 26% loss at 37°C. (166)

Losses of toluic and benzoic acids, used for monitoring exposure to xylene and toluene, respectively, were less than 20% during one month at 4°C. However, if the urine samples were stored unhydrolyzed, ie., the chemicals were in the form of glycine conjugates of the acids (hippuric and methylhippuric acids), the losses were much more pronounced. (46) However, when urine specimens were stored at -20°C, hippuric and methylhippuric acids remained stable for at least a year, and trichloroacetic acid and phenol for at least 6 months. (Pekari K, unpublished)

Trichloroethylene from blood specimens was lost to air, and to silicon rubber. However, if the specimens were acidified, and methylated with dimethyl sulphate immediately after collection, the methyl ester remained stable for at last 48 h. (102)

Acrylonitrile was rapidly lost, probably by metabolism, so that already one hour after the sampling the concentration in spiked rat blood had fallen by approximately 20%. (53)

Evidently, the stability of chemicals varies greatly; the matrix may have a pronounced effect on the storage possibilities. For each chemical analysed, the stability has to be verified.

Precipitation and adsorption. Precipitation is a problem which is especially prominent with urine samples. Urine, when voided, is often an oversaturated solution of certain components, eg. urates and phosphates. In addition, it contains cells and cell conglomerates which may act as centres for crystallisation. Therefore, urine, although it might be clear when voided, tends to precipitate on storage. Phosphates tend to precipitate in alkaline, and uric acid in acid urine. Cooling, or warming, may cause additional precipitate formation. Bacterial infection in the urinary tract may increase the tendency to precipitation, and cause turbidity even in a freshly voided urine specimen. Trace elements in the urine may be coprecipitated with other elements, or adsorb onto the surface of the precipitates.

When ^{63}nickel was added to urine samples immediately after voiding, nickel was found to be adsorbed on the precipitate that formed. This loss of nickel was pH-dependent: at pH 1 less than 1 % was lost, whereas at pH 6 upto 6 per cent were lost (149). When urine was stored at pH $\leq$2, no loss of cadmium on to the forming precipitate was seen. (151)

In a study on the storage of mercury in urine, Trujillo and coworkers (158) noticed that a full recovery of added mercury (1 mg/L) could only be achieved, if the specimen was vigorously shaken before analysis. A variety of preservatives was tested, but none of them could prevent this loss of mercury, although adsorption onto vial surfaces could be completely prevented (see Adsorption loss) (158). Years earlier, Lindström (90) had shown that centrifugation of urine decreased its mercury content.

Cornelis and collaborators (33) reported that arsenic, copper, antimony, chromium, mercury, selenium and zinc were concentrated in the precipitate upon storage for 2 days of acidified urine, whereas manganese, cobalt, caesium and rubidium remained in the supernatant fraction. The extent of losses was not given. Molin Christensen and coworkers (101) obtained similar results for urine cobalt, when they used either AAS of filtered urine, or anodic stripping voltametry after wet digestion.

Marked differences thus exist between losses of different metals on precipitates in urine. This loss must always be accounted for when sampling urine specimens. Precipitation is a factor to be remembered also when preparing quality control specimens in a urine matrix: the homogeneity of the quality control specimens has to be investigated carefully.

Adsorption on vial surfaces. Adsorption of metal cations on to surfaces of different types of glass, or plastics from distilled, fresh, and even sea water is a well recognized problem. (cf. 31,34,131,132,139,140,141,152, 158,159,160). Acidification of the specimen has in general been used as means of prevention of adsorption (31,34,131,139,141,144,145,152,159).

Much less data are available on the adsorption of elements onto surfaces from blood or urine samples. Stoeppler (149) did not detect any loss of added ^{63}Ni from urine samples onto polyethylene container walls. We

stored urine samples spiked with small concentrations of chromium or nickel for 6 months at 4°C, and did not see any loss of the metals (Kiilunen et al, to be published). The IUPAC reference method for nickel in urine calls for acidification of urine quality control samples with nitric acid and storage in polypropylene tubes with a screw-cap at -20°C (22). No adsorption of cadmium onto container walls (type not specified) was seen from urine acidified to pH<2 (151).

No change was seen in the concentration of cadmium or manganese in blood during 5 days in Vacutainer tubes (116). Polypropylene tubes were found to be suitable for storage of serum for aluminum analysis, whereas Vacutainer (glass) tubes gave rise to either increases (leaching) or losses (adsorption) in the aluminum content (18). Leung and Henderson (88) also considered polypropylene tubes to be suitable for storage of serum specimens for aluminum analysis. Controversial results have been reported on the stability of blood samples for lead analysis: Boone and coworkers (19) stored blood samples (bovine blood) collected from cows dosed with lead, in red stopper Vacutainer tubes at -20°C for 4 months and detected no decrease in the concentration of lead. The results were averages of the analyses from 113 different laboratories, and the mean concentrations studied were 170, 700, and 900 µg/L. On the other hand, de Haas & de Wolff (67) reported a 20 % loss of lead in heparinized Venoject tubes; this loss was completely prevented by silanization of the tubes.

Nackowski and coworkers (108) detected loss of lead due to surface adsorption in blood samples stored in Becton-Dickinson lead-free Vacutainers for a week. Moore & Meredith (105) investigated the disappearance of radioactive lead from (water and) blood at concentrations of 0.6 µmol/L and 1.9 µmol/L onto polystyrene, polypropylene, and Pyrex and soda glass vials. When stored at 0°C, no loss of lead could be detected within 29 d; in some cases clotting of blood prevented proper analysis after 2 weeks. At 20°C, there was a loss of appr 28 % in the sample stored in soda glass vial, whereas for all other vial types, the loss was less than 4 %. Again, clotting of blood proved to be the limiting factor for satisfactory analysis.

The results of Moore and Meredith are in striking contrast with those of Meranger and coworkers (99) who studied the storage of swine blood for trace metal analysis. A substantial loss of lead was detected after 2-5 days, when blood was stored in polyethylene, polypropylene, polystyrene, polycarbonate, or soda or Pyrex glass containers, irrespective of the storage temperature (22, 4, -10, -70°C). Cadmium remained stable in similar conditions for appr 1 month (99). The rapid loss of lead could be a peculiarity of swine blood, as the same authors more recently reported that in human blood not only cadmium but also lead remained stable for 2 weeks at 4°C in polyethylene and polypropylene vessels; in polycarbonate tubes at -10°C the samples could be stored for 2 months (153).

Anand and Ducharme (7) showed, that the concentration of serum chromium remained unchanged for less than 4 days at room temperature, less than 3 weeks in a refrigerator, but more than 18 months at below -10°C in polyethylene or polycarbonate tubes. However, the concentration of chromium used was 50 µg/L, and the result therefore has probably little relevance when true serum specimens are stored.

A solution of cobalt 0.3 µg/L in appr 0.1 molar salt solution, at a neutral pH, was stable for 28 days in polystyrene tubes (14).

Although the adsorption of trace elements on container surfaces from biological specimens has not been thoroughly studied, it seems that this process is not equally important as it is in the storage of water specimens.

Published data on the surface adsorption of organic toxic chemicals is even more scanty than of trace elements but it is general knowledge that hormones for example, which have only limited solubilities in water, tend to disappear from dilute aqueous solutions. Recently Rick and coworkers (123) reported that pentachlorophenol was adsorbed from urine samples onto plastic (unspecified) and metal (unspecified) surfaces resulting in 10-30 % losses. In a glass container the specimens remained stable for one week in a refrigator; three week-storage in a freezer resulted in a loss of 20 %. On the other hand, the more readily water soluble phenol in urine remained stable for at least one week in the refrigerator in plastic vials, and in

frozen state for at least one year (123).

CONTAMINATION

Contamination is by far the most important source of error in the analysis of trace elements. Trace elements are ubiquitous in the earth's crust, and tend to enter the samples in all phases of the performance of the measurement. The increasing sensitivity of analytical methods has resulted in the use of ever decreasing amounts of the specimens, and therefore, to ever decreasing amounts of the analyte in question. Thus the effect of even a µg-level of contamination is catastrophic. Recognition of contamination has also changed our views on the true concentrations of many trace elements in biological samples, to the extent that the prevailing concept of actual concentrations of some metals in serum or urine is no more than one thousandth of what was thought to be the truth barely 10 years ago (see 170). Contamination may be derived from the ambient air in the work place, or in the laboratory; from the skin of the subject or the collector of the sample; from specimen containers; from additives (anticoagulants, preservatives), and reagents used in the analysis, as well as from parts of the analytical instrumentation, such as pipette tips or neutron activation vials.

In this presentation, contamination during the analysis will not be dealt with; thus questions related to air conditioning in the laboratory, or to the purification of reagent-chemicals all fall outside the scope of this review; the reader is referred to the modern analytical literature.

Workplace air and skin. Contamination from workplace air causes the most drastic errors in measurements of toxic chemicals in body fluids. The reason is the high concentrations of the chemicals in workplace air, which tend to be orders of magnitude higher than, e.g. the air in the laboratories.

Contamination in the workplace also affects analyses of organic chemicals that are otherwise not equally prone to error due to contamination. This is because chemicals may be present in dust where the samples are collected. Chemicals may show high concentrations on the clothes of the worker and on his skin.

In addition to air-borne dust, contamination on the skin may result from sweating: Hohnadel and coworkers (72) detected high concentrations of nickel, copper, zinc and lead in sweat collected during a sauna bath. They also cite another study (not available to us) indicating that in addition, aluminum, manganese, molybdenum and tin are excreted in sweat. The absolute quantities found, 50 to 500 µg/L, of course are not easily interpreted as to their significance as a source of contamination. However, it is to be borne in mind that as water from sweat evaporates, the quantities remaining on the skin may be rather high. Another point to remember is that it is rather customary to explore the vein in the forearm by lightly pressing the skin with a finger. Therefore it is not only the skin of the subject, but also the hands of the person collecting the specimens that may be a source of contamination.

The skin has to be cleaned thoroughly before drawing blood samples for toxicological analysis. Washing with 0.1 mol/L hydrochloric acid, followed by rinsing with ethanol, removed lead contamination from the skin. Washing with only ethanol or ethanol and water, was not sufficient for this purpose. (75) In a study of plasma lead, Everson and Patterson (48) washed the skin successively with soapy water, alcohol, acetone, hydrochloric acid, and low-lead water.

The risk of contamination from the work place air is even greater when urine specimens are collected. They should not be collected in the workplace, but at a separate site, and only after making sure that no dust from the clothes of the worker may reach the specimen container.

Needles. By activating disposable stainless steel needles with neutrons, and analyzing the resultant radioactivity in blood specimens drawn from plastic tubes with these needles, Versieck and Speecke (171) and Versieck and coworkers (168) showed that large amounts of chromium, nickel, cobalt and manganese were leached in the first 20 mL of the blood. With a similar

technique, Lakomaa (84) demonstrated that specimens of cerebrospinal fluid obtained through conventional needles could not be used for assessing chromium or nickel concentrations because of contamination from the needle. Damage caused by the activation process did not explain the findings (168). Recently, Kumpulainen et al (83) also showed that concentrations of chromium in the blood obtained through disposable stainless steel needles was 4 times higher than that in blood obtained through a plastic cathether. No contamination of manganese was seen in the blood drawn through a polypropylene catether. (168). The IUPAC reference method for the determination of nickel in serum calls for for blood specimen collection by means of Teflon-polyethylene intravenous cannula (22). Platinum needles have been used with advantage in sampling for nickel (35).

The concentration of aluminum in blood collected through plastic cannula was not different from that of blood collected through disposable stainless steel needles. (18,52,55,60). However, Parkinson and coworkers (115) recently reported that stainless steel venipuncture needles they used were randomly contaminated with aluminum.

Contamination of blood specimens with metals, especially with nickel, chromium, cobalt and manganese, from needles seems to be an established fact. However, it is not clear, whether it has significance at concentration levels at and above the upper reference limit of the general population. This point urgently needs clarification.

Contamination from anticoagulants and preservatives. If a chemical is to be analyzed in whole blood, coagulation of the specimen has to be prevented. Otherwise a representative sample of the blood may not be obtained. Anticoagulation may be achieved by either heparin, or by removing calcium from the blood sample. With either method, anticoagulation is not necessarily long-lasting. Eventual coagulation may well be the reason for not being able to do a proper analysis, even though the analyte itself may be stable. (105)

Information on the contamination resulting from the use of anticoagulants is very scanty. Cavalleri and Minoia (28) calculated that the heparin which they used would add 0.59 + 0.1 ng lead/ 5 mL of blood. On the other hand, Everson & Patterson (48) found that more than 99% of the lead in plasma was actually derived from heparin. It seems that the lead in heparin does not contribute significantly to the concentration of lead in whole blood, whereas it is evident that for lead in plasma the reverse might be true, as the concentration of lead in plasma is very low (28,29,38,48,96). Lead in blood, however, is exceptional amongst trace elements because of its high concentrations; for most other metals the concentrations in blood are very much lower (cf. 170), and thus significant contamination from heparin is possible.

Other anticoagulants act by scavenging calcium, such as oxalate, EDTA and fluoride. Although analytical data are not available, it would seem that these other anticoagulants are even more probable as sources of contamination, as they tend to bind not only calcium but also other metals. Moreover, their purification is difficult.

Hydrochloric and nitric acids have been used as preservatives for urine specimens for mercury and cadmium analysis (eg 57,151), and for analysis of metals in general in water samples. They - especially HNO_3 - have an important role in the present day electrothermal atomic absorption analysis methods, too. Kuehner and coworkers (82) analyzed the contents of trace metals in reagent grade and commercial high purity acids. The concentrations of lead, cadmium, nickel, and chromium were generally in the order of 1-100 ng/g, which is incompatible with analysis of trace elements in biological (or water) samples. High purity acids were not invariably better than reagent grade acids. The situation might be different at present, because recently it was reported that commercial ultrapure hydrochloric acid was equally acceptable as the acid prepared by sub-boiling distillation in the analysis of chromium in urine (167). Ultrapure commercial nitric, sulphuric and perchloric acids without further purification were also found suitable for nickel analysis in urine (22) and so were ultrapure perchloric, nitric and acetic acids for the analysis of lead and cadmium in urine (59). Reagent grade hydrochloric and nitric acids, though, still contained nickel at concentrations in excess of 50 µg/L. (110) Aristar grade nitric acid contained 11-32 µg/L aluminum. (115)

Contamination from glass and plastic ware. Glass (cf 130) and various plastics (78,104,130) contain varying amounts of practically all elements, and these may leach into water, acids, as well as blood and urine specimens. It seems that no commercially available container should be used for storage of such specimens for trace element analysis without prior cleaning. A variety of cleaning methods has been devised. (78,82,86,100, 104,130) In a widely used method (104), the containers are first soaked in hydrochloric acid for one week, then, after rinsing with distilled water, they are soaked for another week in nitric acid, and then, after rinsing, for several weeks (or until they are used), in the purest water available. This procedure, however, requires large numbers of containers, and space for the soaking operations, and is somewhat impractical for routine use. In a thorough study, Laxen and Harrison (86) compared 13 different published cleaning methods. These included different concentrations of nitric, hydrochloric and perchloric acids, and combinations of them, as well as use of nonionic detergents, and also rinsing with distilled water only. The conclusion was that for analysis of zinc, cadmium, lead and copper in fresh water, soaking in 10% nitric acid for 48 h was the most appropriate method of cleaning.

Evacuated blood collection tubes (Vacutainer^R, Venoject^R, Monoject^R, etc), and especially their rubber stoppers, have been notorious for their contamination with metals, which was first recognized with lead and cadmium. At present, many manufacturers market evacuated tubes specifically destined for lead, or more general trace element analysis but there may be differences between the different brands.

Guillard and coworkers (64) detected a 3.5 fold increase in the aluminum content of serum specimens stored in conventional Vacutainer^R tubes. Oster (114) saw an aluminium contamination of < 1 µg/L from Vacutainer tubes (type not specified). No aluminum contamination was found after 24 h in trace metal Vacutainers, and even after eight days, the increase in aluminum content was 15%. (64) Red top (trace element) Vacutainers were not found to give rise to significant aluminum contamination in two studies (18,88), and a similar finding was obtained with Monoject (Sherwood) (60) as well as Monovette (Sarstedt) tubes (143).

Hellman and coworkers (69) detected marked lead contamination in standard Vacutainer tubes, but none in "minimal lead" tubes. Blood lead concentrations, measured after 24 h storage in Radiometer heparinized tubes, or Radiometer heparinized tubes with a mixing flea, were on average 23 and 58 % higher than after storage in Becton-Dickinson minimal lead Vacutainer tubes, whereas samples collectd in Natelson tubes and centrifuged and stored in Spinco microcentrifuge tubes, showed similar lead concentrations as the minimal lead Vacutainers (75). No lead leach could be detected into 1% HNO_3 from Becton-Dickinson or Abbot-Labtube lead-free evacuated tubes, whereas lead was leached from Venoject^R EDTA and oxalate fortified tubes, from one variety of EDTA Vacutainer, and sodium fluoride fortified tube from Abbot-Labotube (108). Nise and Vesterberg (111) did not find any increase in blood lead after storage for three days at 22°C, and thereafter for 4 days at 4°C in Venoject T-200KA, or Vacutainer 3200 KA, or Vacutainer L3200 x F313, or Nunc 1203/3, or Vitrum heparin tubes. In a recent study reported in an abstract (89), workers from Becton-Dickinson reported that the lead contamination from Vacutainer brand trace metal tubes, after leaching with 0.1 mol/L nitric acid for an unspecified time was less than 0.70 µg/L

Low lead Vacutainer tubes introduced a marked leaching of cadmium into 1% HNO_3 within one day. Even other evacuated tubes (Venoject EDTA, potassium oxalate, Vacutainer EDTA, Labtube) showed a cadmium leach into human blood within 1-2 weeks, although no leaching could be detected into nitric acid. (108) Nise and Vesterberg (111) also demonstrated a cadmium leaching from Becton-Dickinson tubes of the series 3200 into 0.01 mol/L nitric acid in 5 days, and also increased concentrations of cadmium in blood stored in these tubes. Red caps used for syringe closure leached cadmium into blood to the extent that the analytical result overestimated the true concentration by a factor of 3.5. (150) No leach of cadmium was seen from Becton-Dickinson brand trace metal tubes into 0.1 mol/L nitric acid (89); no increase in the concentration of cadmium in blood stored in Vacutainers was seen (116). The latter two studies were in an abstract form; they also reported that the leaching of manganese into 0.1 mol/L nitric acid was less than 70 µg/L, and not detectable to blood in 5 days at 4°C. The results of Lecomte and

coworkers (87) suggest that even nickel might leach from Vacutainer^{R} tubes to serum samples.

It seems advisable that the laboratory doing the analysis should provide the specimen containers; this helps to diminish contamination risks. Commercial evacuated tubes may introduce contamination to blood specimens. Although this risk seems smaller if dedicated trace metal brand tubes are used, absence of contamination should be verified before starting use of commercial tubes.

Loss of water. Loss of water by evaporation results in apparent increases in the concentrations of the chemicals studied. The water loss may be through the container walls, or through leaky closures; therefore the results that one obtains may be quite variable from one container to another. Generally, errors introduced by water loss are minor, and do not affect everyday routine analyses. However, in specimens with long storage times (especially quality control materials !) such losses may become significant. Annual losses of water from polyethylene, polypropylene, high density polypropylene, Teflon-FEP, and glass containers were less than 0.5% (36,104). Losses from polyvinyl chloride was appr. 0.5%, those from polycarbonate appr 2% and from polymethylpentane 1% annually (104)

Redistribution of the analyte. The concentration of manganese is 23 times higher in erythrocytes than in plasma (168). The ratio for lead in still debated, but is probably even higher (28,29,38,48,96); for zinc it is appr. 10. It is thus evident that destruction of cells leads to gross elevation of the concentration of such elements in plasma/ serum. Plasma may be separated from cells more rapidly than can serum, and the procedure is more gentle toward the cells. Therefore, plasma is to be preferred over serum, when elements with unequal distribution in blood are analyzed, although addition of anticoagulant adds a risk of contamination (see above). Hemolysis may give rise to analytical interferences eg. by liberation of considerable amounts of iron into the serum: iron interferes with eg the analysis of nickel by electrothermal atomic absorption (22).

Storage of specimens for mercury analysis. Mercury solutions, in water, and in urine, are notoriously unstable (cf. 12,20,30,31,34,49,57,63,68,90,91, 92,95,98,113,132,154,156,158). The mechanisms of such losses seem to be manifold: evaporation, adsorption on container walls, and precipitation (see above). Even diffusion through polyethylene has been suggested. (98) It seems evident that a prerequisite of loss by adsorption and evaporation is the reduction of mercury (II) to mercury(I), and subsequent disproportionation to yield metallic mercury Hg^{o} and Hg^{2+} (157). Metallic mercury is then easily evaporated, and may also be adsorbed on to container surfaces. Reduction of bivalent to monovalent mercury proceeds in many biological systems. Thus Magos and coworkers (95) isolated three strains of bacteria from tap water that catalyzed this reaction. Toribara and coworkers (157) state that it is difficult to find a biological system that does not catalyze this reduction. Acidification by for example with nitric acid has been the classical method of stabilizing low level mercury solutions (57). In the study by Trujillo and coworkers (158) mercury was apparently lost from urine samples with a spiked concentration of 1 mg/L. However, this loss was completely accounted for by precipitation of mercury: reanalysis after shaking brought the concentrations of mercury to the original value. The level of mercury studied here was of course not really relevant and at least with water samples preservation by means of acidification has not given unequivocally positive results (49), and alternatives have been sought; for example, the addition of strong oxidizing agents such as potassium permanganate or potassium dichromate (31,49,92,157). Christian and Ingle found that a combination of 5 % HNO_3 and 0.01 % $K_2Cr_2O_7$ was able to stabilize aqueous solutions of 1 µg/L mercury for at least 6 days. One per cent HNO_3 with 16 µg/L Au(III) had the same effect. Gold is used to stabilize mercury in the National Bureau of Standards' aqueous reference material. It should be noted however that the combination of nitric acid with potassium dichromate destroys methylmercury. Therefore, if speciation of mercury is intended, the samples may not be preserved with this mixture. (113) Trujillo and coworkers (158) showed that potassium persulfate was capable of preserving urinary samples for mercury analysis when the concentration of mercury was 1 mg/L. As the addition of a solid would be very convenient in routine practice, it would be worth while verifying this finding at low mercury concentrations.

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