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Meeting Report

New applications of biological monitoring for environmental exposure and susceptibility monitoring. Report of the 7th International Symposium on Biological Monitoring in Occupational and Environmental Health

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Abstract
Validated biological monitoring methods are used in large-scale monitoring programmes involving determination of ubiquitous environmental pollutants such as metals and pesticides. Some programmes focus on children's exposure, and policies to prevent adverse health effects. Most of these initiatives are aimed at characterizing trends. Some of these programmes are designed to investigate the role of certain exposures in disease. Fewer new biological monitoring methods were presented during the present meeting than in previous meetings. All of these new methods used mass spectrometric-based detection and quantification. There is an increasing use of biomarkers to study genetic polymorphisms of enzyme systems involved in both toxification pathways and metabolite conjugation and DNA repair. At the meeting a discussion was started that could lead to a further harmonization of the scientific fundaments of the use of biological monitoring in occupational health with possible value also for applications in the field of environmental health.

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Introduction

Previous meetings were in Kyoto (1992), Parma (1994), Helsinki (1996), Seoul (1998), Banff (2001) and Heidelberg (2004). A report of the Heidelberg meeting appeared in *Biomarkers* (Scheepers & Heussen 2005). This year the meeting was hosted by Dr Sheng Wang of Peking University Health Science Centre. The meeting was prepared by the Scientific Committee on Occupational Toxicology (SCOT) of the

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International Commission on Occupational Health (ICOH). The venue of the meeting was the Jiuhua Resort and Conference Centre just 30 km north of Beijing.

Reappraisal of the merits of biological monitoring

The scientific programme was opened by a keynote speech from Dr C. Viau (claude.viau@umontreal.ca) of the University of Montreal, Canada in collaboration with Dr M. Manno of the University of Naples Federico II, Italy, addressing some fundamental problems related to the use of biological monitoring, including: not all biomarkers are the same; there are different biomarkers for different aims (both research and routine). Not all biological limit values are the same and there are some important methodological issues to consider when establishing these values, in particular in the context of decreasing occupational exposure. There are also a number of ethical issues that should be addressed when applying biological monitoring. Most of these issues are covered in a position document of which a preliminary draft document was included in the conference proceedings. This document will be submitted to the ICOH and will possibly be studied at a later stage. The most important issues discussed concerning the position paper were: harmonization with other existing documents and with bodies like the ICOH; interactions of occupational and environmental applications; and the need to be precise about the terminology used.

Some interesting polymorphisms

Metallothioneins (MTs) are a family of four isoforms of metal-binding proteins in serum. A genetic polymorphism causes differences in activity of at least one isoform: a high MT-activity phenotype resulted in a protective effect following exposure to cadmium (Nordberg et al. 2007). This MT phenotype can be determined as metallothionein mRNA (MTmRNA) in peripheral blood lymphocytes. A low level of MTmRNA is associated with increased β_2-microglobulin, a urinary biomarker for tubular kidney damage. Another susceptibility marker is the occurrence of auto-antibodies against MT (MTab). A positive MTab status is associated with an increase of tubular effects as indicated by an increased β_2-microglobulin, presumably caused by low levels of cadmium.

A further polymorphism was observed in a study presented by Dr P. Manini (paola.manini@unipr.it) of the University of Parma, Italy. The study focused on human 8-oxoguanine DNA N-glycosylase 1 (hOGG1), an enzyme involved in the repair of oxidative DNA damage (removal of 8-oxoguanine opposite to cytosine by base excision repair). The impact of this polymorphism was studied in a group of workers exposed to styrene and a group of non-exposed reference subjects. Dr Manini used urinary metabolites mandelic acid, phenylglyoxylic acid and 4-vinylphenol as markers for styrene exposure and 8-oxo-2′-deoxyguanosine (8-oxo-dGuo) in white blood cells and 8-oxo-guanine (8-oxo-G) in urine as markers of oxidative DNA damage. Of the urinary metabolites 4-vinylphenol showed the strongest association with indicators of oxidative damage. Subjects with the homozygous wild-type genotype (hOGG1WW) had lower values of 8-oxo-dGuo in white blood cells but higher concentrations of 8-oxo-G in urine than subjects carrying at least one variant hOGG1 allele. A remarkable pattern of oxidative DNA damage was observed with the

most heavily exposed workers showing the lowest blood levels of 8-oxo-dGuo but also the highest urinary 8-oxo-G levels (of all workers). This complementary pattern of damage is consistent with induction of enzymatic base excision DNA repair by styrene exposure.

Paraoxonase (PO) exhibits a polymorphism in humans with individuals classed in three distinct phenotypes: slow, intermediate and fast metabolism. The distribution in the Caucasian population for these phenotypes is 47:40:13. The PO phenotype was measured as the ratio of PO and aryl esterase activity according to Williams et al. (1993). Dr J. Edwards (john.edwards@flinders.edu.au) of the University of Flinders (Adelaide, Australia) measured PO activity by the conversion of paraoxon to *p*-nitrophenol and aryl esterase activity by the conversion of phenylacetate to phenol. Cholinesterase (ChE) inhibition was measured as a biomarker of effect. A correlation was found between ChE inhibition and the ratio of paraxonase/aryl esterase activity. Individuals with a slow metabolism may be at greater risk for toxicity of OP-ester pesticides because of accumulation of OP insecticide. An overview of polymorphisms that were studied using biomonitoring methods is presented in Table I.

Metal exposures

Dr G. Nordberg (gunnar.nordberg@envmed.umu.se) of the University of Umeå, Sweden performed studies on combined exposure to cadmium and arsenic in the Chinese general population. An important source of exposure is the use of coal for preparation of smoked fish and meat. A more pronounced effect of cadmium/arsenic co-exposure on renal damage was observed compared with the toxic effect expected for each of the metals following a single exposure situation. This effect has also been observed in animal studies. More field studies are needed to confirm this preliminary finding.

Monitoring programmes

The Centers of Disease Control (CDC) in Atlanta, USA have been conducting a large study known as the National Health and Nutrition Examination Survey (NHANES). Since 1999, 5000 individuals have been studied. Previous reports have been published in 1999/2000 (25 chemicals), 2001/2002 (125 chemicals) and 2003/2004 (300 chemicals). A fourth report will appear in separate contributions in scientific journals in 2007 and 2008. It should be noted that the study population includes workers with possible occupational exposure to these chemicals. The datasets can be downloaded and can be linked to other datasets such as mortality data. More information about this programme can be found on www.cdc.gov/nchs/nhanes.htm

Dr M. C. R. Alavanja (alavanjm@mail.nih.gov) of the National Cancer Institute in Bethesda reported on another large study in the USA known as the Agricultural Health Study (AHS) which started in 1993 and involves 89 658 pesticide applicators in Iowa and North Carolina. The aim of this study is to find out if pesticide exposure is a risk factor in cancer. For this prospective cohort study self-administered questionnaires and computer-assisted telephone interviews were used to collect exposure data. These data were incorporated into an exposure algorithm and expressed as exposure scores (Dosemeci et al. 2002). Biological monitoring was used to validate this method of data collection, using 2,4-D or chlorphyrifos by

Table I. Studies addressing the role of specific genetic polymorphisms.

Polymorphism/ genotype	Phenotype/ enzyme function	Determined by	Population	Exposure/ substrate	Biomarker of exposure	Biomarker of effect/ bioactivation	Presented by:
HOGG1	Enzymatic DNA excision repair	Metabolite excretion by LC–MS/MS	Plastic workers	Styrene	4-vinylphenol in urine	8-oxo-G (plasma)/ 8-oxo-dG (urine)	P. Manini et al.
NAT2	Hepatic acetylator enzyme (phase 2)	7 specific SNPs by RT-PCR	Rubber workers	o-Toluidine	o-Toluidine in urine	o-Toluidine haemoglobin adducts	T. Weiss et al.
MT	Metallothionein synthesis	mRNA expression in peripheral lymphocytes	Cadmium workers and general population	Cadmium	Cadmium in urine	β_2-microglobulin as indicator of renal tubular damage	G. Nordberg et al.
AhR, CYP1A1, CYP1B1	Hepatic oxidative enzyme system (phase 1)	RT-PCR	Fire-fighters	Dioxins	Total-TEQ	–	J.A. Grassman et al.
AhR, CYP1A1, CYP1A2	Hepatic oxidative enzyme system (phase 1)	Antipyrine metabolism measured by HPLC	Fire-fighters	Dioxins	Total-TEQ and 3-hydroxymethylantipyrine	–	Y.I. Chernyak et al.
GSTM1	Glutathion conjugation (phase 2)	Determination of urinary metabolite excretion by LC–MS/MS	Varnish workers, reinforced plastic workers	Styrene	Mercapturic acids (M1, M2)	–	S. Fustinoni et al.
PON1/PON2	Paraoxonase activity	Metabolite excretion by UV-absorption	General population	Paraoxon	*p*-Nitrophenol	Inhibition of cholinesterase activity	J. Edwards et al.
–	Arylesterase activity	Metabolite excretion by UV absorption	General population	Phenyl acetate	Phenol	Inhibition of cholinesterase activity	J. Edwards et al.

86 applicators. The exposure scores were statistically significantly correlated with urinary metabolite concentrations. Including pre-exposure urine concentrations increased these correlations. It was found that the questionnaire-based exposure scores reduced exposure misclassification and thus improved the study. Preliminary findings suggest that exposure to 15 pesticides is associated with cancer risk. Since none of these substances have genotoxic properties it must be assumed that epigenetic mechanisms are involved.

Germany has its national German Environmental Survey (GerES). This study has been carried out repeatedly since 1985. The study population was recruited from all over Germany. In the last edition of this programme (GerES IV) 1790 children ranging in age from 3 to 14 years were recruited. Almost all the children were non-smokers. Exposure to environmental tobacco smoke (ETS) was assessed by urinary cotinine. Urinary levels were $<2\ \mu g\ L^{-1}$ if neither of the parents smoked, $2.6\ \mu g\ L^{-1}$ if one of the parents smoked, and $4.8\ \mu g\ L^{-1}$ if both parents smoked. The results of this study indicated that children had higher exposure if the mother smoked than in those cases of paternal smoking.

The German GerES IV study (performed in 2003–2006) showed a decrease of about 50% in blood lead levels observed in children compared with data from GerES II (1990/92). A. Conrad (andre.conrad@uba.de) from the Federal Environment Agency of Dessau-Roßlau/Berlin showed that lead concentrations were higher in boys than girls. Higher levels were observed in children with a low social economic status. This dataset also showed a gradual decrease of blood lead with four strata of age (3–5, 6–8, 9–11 and 12–14). In a discussion with the audience the source of lead exposure was discussed. Lead in petrol was banned in Germany in 1971. It is speculated that the gradual decrease with age could be the result of (1) slow excretion after uptake of lead during pregnancy and lactation, (2) change in uptake to body weight ratio or (3) finger shunt (so-called pica behaviour) uptake of lead from pigments (banned from indoor use in Germany since 1980) or house dust. A similar decrease in lead levels with age was observed in the NHANES study of CDC, Atlanta. The GerES IV dataset will become available for analysis as a scientific use file.

In Europe another large-scale human biomonitoring initiative is in a preparation phase. During an EU project (ESBIO) the outlines for a large-scale infrastructure for human biomonitoring were developed. Twenty-four of the 27 EU member states will be involved with some 54 participating institutes in a new 'Consortium to Perform Human Biomonitoring on a European Scale (COPHES)', explained Dr L. Bloemen (lbloemen@exponent.com) from one of the participating groups (Exponent). Children of 6–11 years old will be recruited together with their mothers. The project will have two phases. In the first phase a limited number of biomarkers will be involved (lead, methyl mercury, cadmium and cotinine). The second phase of the project will cover polycyclic aromatic hydrocarbons (PAHs) and some new interests like brominated flame retardants, phthalates and organotin compounds. The possible use of saliva as an alternative non-invasive biological medium will be studied. During the discussion one of the coordinating scientists explained that this project will attempt to give attention to policy-related issues concerning environmental health. This requires that, in addition to biomarker data, efforts must be made to collect data, on the lifestyle characteristics and environmental factors of the study population.

New methods

An overview of the new and improved methods for biomonitoring is given in Table II. Dr Schettgen (tschettgen@ukaachen.de) of Aachen University, Germany reported on the development of a new method for simultaneous determination of two urinary mercapturic acids: S-benzylmercapturic acid (SBMA), a metabolite of toluene and S-phenylmercapturic acid (SPMA), a metabolite of benzene. The method is based on the analysis on an automated multidimensional liquid chromatography-electrospray ionization–tandem mass spectrometry (LC/LC/ESI–MS/MS) system. The pretreatment of the samples was limited to addition of formic acid. Each run takes approximately 23 min. Fivefold deuterated SPMA (d_5-SPMA) was used as an internal standard. The method is very sensitive with a limit of determination (LOD) of 0.5 $\mu g\, l^{-1}$ for SBMA and of 0.05 $\mu g\, l^{-1}$ for SPMA. With this sensitive method it was feasible to quantify background exposures in the non-smoking general population. Dr Schettgen expected these biomarkers to reflect recent exposure (of the previous 12 h). In 32 non-smokers, average SBMA and SPMA values were 8.0 and 0.12 $\mu g\, l^{-1}$, respectively. For smokers these values were: 12.1 and 1.2 $\mu g\, l^{-1}$, respectively. For SBMA the difference between smokers and non-smokers was not statistically significant. This raised some questions as to the source of toluene exposure. Apparently, there are other sources (than smoking) that contributed to the observed SBMA levels.

Dr Schettgen also reported on the LC–MS/MS analysis of a mercapturic acid derived from ethylene oxide exposure (N-acetyl-S-(2-hydroxyethyl) cysteine, HEMA) in urine of workers involved in industrial production of emulsifiers and detergents. Urine was pretreated off-line using solid-phase extraction (SPE). Only a small but statistically significant difference in excreted HEMA levels was observed between workers (an average of 5.4 $\mu g\, l^{-1}$) and controls (an average of 3.1 $\mu g\, l^{-1}$).

Ms K. Jones (kate.jones@hsl.gov.uk) from the Health and Safety Laboratory in Buxton, UK developed new analytical methods for the primary metabolite of the carbamate pesticide pirimicarb (2-methylamino-5,6-dimethyl-4-hydroxypyrimidine, MDHP). Two new methods were developed and validated: an LC–MS-based method and an immunoassay (ELISA). For LC–MS analysis urine samples were diluted with sodium hydroxide extracted with ChemElut-cartridges and eluted with ethyl acetate containing formic acid (0.1%). Before analysis the eluent was evaporated under nitrogen and the residue was redissolved in the mobile phase (methanol/ammonium formate) of the LC system. The $[M+H]^+$ ion at m/z 154 was used for quantification using an ion trap MS operated in the positive atmospheric pressure chemical ionization (APCI) mode. The LOD of the method was 2.5 $\mu g\, l^{-1}$. Some rather preliminary results showed that a similar LOD of 2 $\mu g\, l^{-1}$ could be achieved using the immunoassay. Only a non-pesticide homologue (not expected in workers) showed cross-reactivity. These methods were validated in a volunteer study involving three males and two females. These subjects received a single oral dose, corresponding to the acceptable daily intake of 0.02 mg kg^{-1} body weight. The interassay coefficient of variation (CV) for the LC method was slightly better than that for the immunoassay (9% and 12%, respectively). Metabolite excretion peaked 2 h after administration and showed a first-order decrease up to 35 h after administration. From this decrease a mean half-life of 3.7 h was calculated. Using total MDHP, an average of 54% of the pirimicarb dose was recovered in urine.

Table II. New and improved biomarkers introduced at the 7th ISBM in Beijing.

Chemical substance	Toxicity	Biomarker	Method	Validation	Presented by
Benzene and toluene	Carcinogenic Neurotoxic	S-phenyl mercapturic acid (SPMA) and S-butyl mercapturic acid (SBMA) in urine	LC/LC–ESI–MS/MS	General population	T. Schettgen et al.
Ethylene oxide	Carcinogenic	N-acetyl-S-(2-hydroxyethyl) cysteine (HEMA) in urine	LC–MS/MS	Workers	T. Schettgen et al.
Pirimicarb	Neurotoxic	2-methylamino-5,6-dimethyl-4-hydroxypyrimidine (MDHP)	LC–MS	Volunteers	K. Jones et al.
Ethylenethiourea	Mutagenic, carcinogenic, immunotoxic, teratogenic	Ethylenethiourea	LC–ESI–MS/MS	Workers and general population	C. Lindh et al.
Acryl nitril	Carcinogenic	N-acetyl-S-(2-cyanoethyl)-cysteine (CEMA), N-acetyl-S-(1-cyano-2-hydroxyethyl)-cysteine (1H-CHEMA)	LC–ESI–MS/MS	General population	E.C. Hartmann et al.
PAH	Carcinogenic	1-hydroxypyrene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 2-hydroxyfluoranthene	HPLC–Flu	General population	T.C. Saowaphak et al.
PAH	Carcinogenic	1, 2-hydroxynaphthalene, 2-, 9-hydroxyfluorene, 1-, 2-, 3-, 4-, and 9-hydroxyphenanthrene, 1-hydroxypyrene, 6-hydroxychrysene and 3-hydroxybenzo[a]pyrene, naphthalene, acenaphtylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[a]pyrene	GC–MS	Workers	L. Campo et al.

Dr C. Lindh of Lund Univerisity in Sweden studied ethylenethiourea (ETU) as a possible biomarker for exposure to ethylenebisdithiocarbamate fungicides (trade name Mancozeb) and for exposure to vulcanization accelerators in the rubber industry. This substance has mutagenic, carcinogenic, immunotoxic and teratogenic properties in experimental animals. Urine and plasma samples were pretreated in a single extraction and derivatization step. ETU was derivatized using pentafluorobenzyl bromide, separated on a C18 column and detected on an ESI–MS/MS system in positive ion mode. Fourfold deuterated labelled ETU (d4-ETU) was used as an internal standard. For quantification the transitions at m/z 463 and 282 was used for ETU and at m/z 467 and 284 for d4-ETU. The LOD was 0.1 $\mu g\ l^{-1}$ in urine and 0.03 $\mu g\ l^{-1}$ in plasma. The method was sufficiently sensitive to detect background exposures in the general population.

ETU was validated as a biomarker in a human experimental exposure to Mancozeb. After exposure, a first-order kinetic pattern was observed with excretion half-lives of 31 h and 16 h in plasma and urine, respectively.

Ms E. Hartmann (eva.hartmann@ipasum.imed.uni-erlangen.de) from Erlangen University in Erlangen-Nürnberg, Germany presented a novel method for determination of two urinary mercapturic acids of acrylonitrile: N-acetyl-S-(2-cyanoethyl)-cysteine (CEMA) and N-acetyl-S-(1-cyano-2-hydroxyethyl)-cysteine (1H-CHEMA). Both metabolites were previously identified in exposure studies of acrylonitrile in rat and mouse. These metabolites were retrieved from urine of smoking workers using SPE followed by LC–ESI–MS/MS analysis. That both metabolites are derived from a common source of acrylonitrile exposure was supported by a high and statistically significant correlation ($r = 0.94$, $p < 0.0001$). Median CEMA levels were 108 $\mu g\ l^{-1}$ (range 9.5–705) and correlated with cotinine suggesting smoking as the primary source acrylonitrile exposure.

In a group of reinforced plastic and varnish workers Dr S. Fustinoni (silvia.fustinoni@unimi.it) of the Fondazione IRCCS Policlinico Ospedale Maggiore and University of Milan, Italy, investigated the exposure to styrene and styrene-(7,8)-oxide. Styrene, mandelic acid, phenylglyoxylic acid, phenylglycine, 4-vinylphenol, N-acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine (M1) and N-acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine (M2) mercapturic acid in urine were higher in the reinforced plastic and varnish workers than in workers not exposed to styrene and styrene-(7,8)-oxide. This difference was statistically significant and inhalation exposure was also positively correlated with levels of urinary metabolite excretion. The level of cysteinyl albumin and haemoglobin adducts of styrene-(7,8)-oxide were elevated but not statistically significantly different from non-exposed workers. Interestingly, a negative correlation was observed between levels of 2-phenylethanol-albumin adducts and urinary excretion of M1 and M2 mercapturic acids. This shows that a comparison of urinary biomarkers and adducts may not be straightforward. Often there is a difference in time-scale: urinary metabolites usually reflect recent exposure, whereas haemoglobin and albumin adducts usually reflect exposure over several weeks or months. There also appears to be an element of competition, suggesting that high levels of urinary excretion of conjugated metabolites (M1 and M2) indicate that reactive intermediates are no longer available for protein adduct formation. This shows how effective the detoxification pathways are in scavenging the reactive metabolites.

A wide variety of exposures to polycyclic aromatic hydrocarbons

Two new methods for the determination of 13 parent PAH (U-PAH) and 12 hydroxylated PAH (OH-PAH) metabolites from urine were presented by Dr L. Campo (laura.campo@unimi.it) of the University of Milan, Italy. OH-PAH were determined by gas chromatography (GC)–MS following enzymatic hydrolysis, liquid/liquid extraction and derivatization, while U-PAHs were measured by headspace-solid-phase microextraction (SPME) followed by GC–MS (Campo et al. 2006). The methods were applied to the analysis of post-shift urine samples from coke-oven workers. Both U-PAH and OH-PAH with four or less rings were measured in all samples, moreover urinary concentrations of PAH metabolites were correlated with concentrations of their respective parent PAH. The results suggest that both OH-PAH and U-PAH are useful biomarkers of exposure to PAH. Moreover the simultaneous determination of several biomarkers permits specific excretion profiles to be obtained which might help in exposure characterization and in better defining of the excretion pattern of the different compounds.

Dr J. Laitinen (Juha.Laitinen@occuphealth.fi) of the Finnish Institute of Occupational Health in Kuopio, Finland reported on a study in fire fighters who were involved in a yearly training of skills needed for rescuing subjects from buildings. This training practice is called 'smoke diving' and is considered the most risky part of the trade. For this training three different set-ups are used: a gas simulator (natural gas burner and artificial smoke), a container and a so-called fire-house. During the training the fire fighting trainers are exposed to carcinogenic agents such as benzene and PAH. The average skin exposures to PAH were 30, 758 and 1164 ng cm^{-2} for the gas simulator, container and fire-house training, respectively. After training in the fire-house the average excretion of 1-pyrenol and 1-muconic acid were 10 nmol l^{-1} and 1.5 μmol l^{-1}, respectively. The fire-fighters' risk of getting cancer is higher than that of the normal population, so these results can explain one part of that, but an important question is if the training conditions mimic the real-life situations.

An entirely different exposure situation is the application of coal tar-derived ointments (CTO) in hospitals. These ointments are used in patients suffering from dermatological diseases (psoriasis or eczema). At Radboud University Nijmegen Medical Centre, an epidemiological study was conducted to find out if these patients are at risk of cancer. Nurses involved in application of these ointments may also be at risk if not well protected. Previous work showed that inhalation of PAH is not likely in this situation. Skin exposure studies showed substantial skin contamination of pyrene and benzo[a]pyrene (median of 33.0 and 16.4 ng cm^{-2}, respectively). In 2006, a controlled field study showed that a 50% reduction in 24 h urinary excretion of 1-OHP could be achieved in nurses if gloves were used. Introduction of new skin protective equipment (acryl gloves and polyethylene fore arm sleeves) in 2007 showed a 50–60% decrease in excretion of 1-OHP when comparing with the old work practice (use of loose-fit polyethylene gloves and no gloves at all when treating children). Sharing biological monitoring results with the nurses raised their awareness of the need to use personal protective equipment.

Dr Saowaphak (scitchty@hotmail.com) of Chiang Mai University presented a study of PAH exposure in the general population, using a comprehensive battery of urinary PAH metabolites (2-hydroxy-naphthalene, 2-hydroxyfluorene, 3-hydroxyphenanthrene and 1-hydroxypyrene). These metabolites were analyzed in a single run using high-performance LC (HPLC) with fluorescence detection (see Chetiyanukornkul

et al. 2006 for more technical details) and deuterated 1-hydroxypyrene as an internal standard. Three groups from the general population were compared: traffic policemen, taxi drivers and inhabitants of a rural village. Quite surprisingly the rural villagers had by far the highest urinary excretion of PAH metabolites and no distinction could be made between smokers and non-smokers. This all pointed to an unexpected source of exposure. It is likely that the high uptake of PAH was related to the habit of food preparation on an open fire in an indoor environment with insufficient ventilation. Possibly also difference in dietary intake between the rural and urban subjects may have contributed.

Pesticide applications and applicators

Dr T. Satoh (satohbri@peach.ifnet.or.jp) from the Ichikawa General Hospital in Chiba, Japan reported on an increased serum β-glucuronidase level following exposure to different OP-esters. β-Glucuronidase (BG) is known for its property to deconjugate glucuronide conjugates that may result in epithelial damage in the gastrointestinal tract or causing enterohepatic recirculation of toxic metabolites. He suggested that OP-esters bind to egasyn, an isoform of carboxylesterases located on the endoplasmatic reticulum in the microsomes of hepatocytes. The substitution of BG by an OP-ester occurred after a 1-day exposure. If rats were exposed for a second day the increase of plasma BG was much lower. After a week this effect disappeared for the most part. This suggests depletion of BG or, (a suggestion made from the audience) the OP-ester–egasyn complex has a life span of several days to release the OP-ester and make egasyn available for binding BG again. When this effect was studied in humans it turned out to be much weaker. Further studies will be needed to clarify the different findings in rats and humans.

Dr B. Rossbach (rossbach@uni-mainz.de) of the University of Mainz, Germany reported a study on the use of permethrin in battle dress uniforms to prevent soldiers of the German Armed Forces from all sorts of vector-borne diseases encountered on various missions abroad. The pesticide is impregnated on the textile before the uniforms are assembled. To evaluate a possible health risk from the uptake of permethrin a human volunteer study was conducted comparing two groups: one group wore non-impregnated uniforms and the other the impregnated ones during office hours for 28 days. Permethrin uptake was assessed by GC–MS analysis of urinary excretion of *cis*-DCCA, *trans*-DCCA and 3-PBA. In the group wearing impregnated uniforms the median urinary excretion of DCCA + 3-PBA was 0.31 $\mu g\, l^{-1}$ on day 0, 31.4 $\mu g\, l^{-1}$ on day 14 and 22.0 $\mu g\, l^{-1}$ on day 28. On day 56 (28 days after the period of wearing the uniform) the level was not yet back to background (1.44 $\mu g\, l^{-1}$). All these values were statistically significantly higher than the values observed in the control group (0.10 $\mu g\, l^{-1}$). Some of the high values were comparable with uptake observed in German pesticide sprayers. Assuming a daily creatinine excretion of 1785 mg for a 21-year-old person weighing 70 kg a body burden of 4 $\mu g\, kg^{-1}$ body weight was calculated, whereas the acceptable daily intake (ADI) is 50 $\mu g\, kg^{-1}$. The study was performed in March at an outside temperature of 10°C. The skin absorption in tropical conditions may be underestimated, since skin absorption is believed to be diffusion (and thus temperature) driven. However, as long as the contribution of different routes of uptake is unknown, it is uncertain what the magnitude of ambient temperature and humidity conditions could be. An interesting observation is that the control subjects

were slightly higher exposed than would be expected, based on German background values. The authors speculate that the control subjects, who were working in the same office, may have inhaled textile dust particles derived from the permethrin-impregnated uniforms of their exposed colleagues.

Hair dyes

4-Aminobiphenyl (4-ABP) can be found as a contaminant in hair dyes. Previous studies showed that elevated levels of DNA adducts were observed in exfoliated breast cells of hair-dye users. Since 4-ABP is known for its bladder carcinogenicity it would be interesting to also study levels of 4-ABP-DNA adducts in exfoliated urothelial cells in urine of users of hair dyes. Urine samples previously collected from women to study exposures at Ground Zero after the Twin Tower disaster will be taken from the freezer to determine these DNA-adduct levels. At that time the use of hair dyes was recorded as one of the items in a questionnaire. So far Dr Talaska (glenn.talaska@uc.edu) of the University of Cincinnati has analyzed 40 of these samples by ^{32}P-post-labelling. Preliminary results show a twofold higher level of DNA adducts in users of hair dyes. Before a more definite conclusion can be drawn, a greater number of urine samples must be analyzed. It will be important to correct this result for smoking since the 4-ABP is expected to contribute approximately 1 ng per cigarette. For this correction Dr Talaska plans to use the urinary 1-hydroxypyrene level. A problem with this metabolite might be that it is expected to be on average only twofold higher in smokers compared with levels observed in non-smokers.

Risk management at the workplace

Several studies showed the relevance of biological monitoring not only in risk assessment, but also in risk management, either by raising awareness of workers and management towards skin exposure, by checking the effectiveness of personal protective equipment or by controlling both skin and inhalation exposure. In one of the case studies presented by Dr H. Heussen (henri.heussen@arbounie.nl) of Arbo Unie (an occupational health service in the Netherlands) the use of breath-analysis of solvents within a paint manufacturing plant helped the management to take further control measures to reduce skin exposure, in addition to measures aiming at inhalation exposure. The importance of skin exposure in manufacturing polyurethanes and epoxyresins using MbOCA (4,4′ methylene bis(2-chloroaniline)) was clearly shown in a study of Dr J. Cocker (Health and Safety Laboratory, Buxton, UK, j.cocker@hasl.gov.uk). Interestingly, 30 years of monitoring showed a gradual decrease of exposure as measured by urinary levels of MbOCA. Another decrease of exposure, in this case for several antineoplastic drugs (AD) was shown by Dr R. Turci (roberta.turci@fsm.it) of the Laboratory for Environmental and Toxicological Testing in Pavia, Italy. Repeated measurements over a 4-year period in hospital employees involved in the preparation and administration of AD showed a dramatic reduction both in the percentage of urine samples with measurable levels of AD and the concentration of the drugs in urine following recommended guidelines on AD preparation and handling, the use of proper devices and protective equipment. Finally, in a study of Dr S. Letzel (letzel@uni-mainz.de) of the Institute of Occupational, Social, and Environmental Medicine/ University of Mainz, Germany urinary aluminium samples were taken from aluminium

welders in the automobile industry between 1995 and 2006. Technical, organizational and individual control measures were implemented to reduce exposure, resulting in internal exposure levels comparable to background exposure – a major achievement keeping in mind that at the beginning of the monitoring programme more than 7% of the aluminium levels in the samples were above the biological exposure limit of 200 $\mu g\, l^{-1}$.

Future challenges in retrospective exposure monitoring

During this meeting it became clear that biomonitoring approaches are more often used in epidemiological studies and ongoing monitoring programmes. As a result of these applications questions are raised concerning the interpretation of biomarkers as indicators of exposure, susceptibility or as markers of a biological effect relevant to the onset of a disease. Biomarkers that are used in this context usually only provide information on exposure. Furthermore, due to the kinetics of uptake, distribution and excretion, most of these biomarkers only reflect recent exposure. If for example a urinary biomarker increases from pre-shift to post-shift, it is reasonable to assume that uptake took place during the preceding shift. For the assessment of exposure over a longer period of time (weeks, months, years) it is not possible to rely only on just one biomonitoring result. To answer those questions one needs to obtain additional data on the work history and, in particular, on the changes of exposure over time. There are some biomonitoring approaches that provide some kind of endpoint that integrates exposure retrospectively over time. These are addition products to proteins such as in haemoglobin or albumin adducts. If there are no mechanisms of enzymatic repair for these products (such as there are in addition products to DNA) and if these products are chemically stable, they can reflect accumulated binding of a xenobiotic or metabolite over several months. In this particular area no new developments were reported during the present meeting, so perhaps this should be seen as a challenge for the future.

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