# Biomonitoring of exposure to environmental pollutants in newborns and their parents in Madrid, Spain (BioMadrid): study design and field work results

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**(Biomonitorización de la exposición a contaminantes ambientales en recién nacidos y sus progenitores en Madrid [BioMadrid]: diseño del estudio y resultados del trabajo de campo)**

## Abstract

In Spain environmental surveillance has mainly relied on measures of selected pollutants in air, water, food and soil. A study was conducted in Madrid to assess the feasibility of implementing a surveillance system of exposure among the general population to specific environmental pollutants, using biomarkers. The project was basically focused on the environment surrounding newborns. Hence, the study population was made up of 145 triplets of pregnant women at around 8 months' gestation, their partners, and newborns from two areas, representing the two main types of urban environments in the region, i.e., the City of Madrid and its outlying metropolitan belt. Multiple biologic substrates were collected from each participant in order to assess the most suitable samples for an environmental surveillance system. The selected contaminants represent the main agents to which a population like that of Madrid is exposed every day, including certain heavy metals, persistent organic pollutants and polycyclic aromatic hydrocarbons, as well as micronuclei in peripheral blood, a commonly used unspecific index of cytogenetic damage. In addition, passive air samplers were placed around subjects' place of residence. This paper reports in detail on the design and response rates, summarizes field work results, and discusses some lessons learned.
**Key words:** Study design. Environmental biomonitoring. Exposure assessment. Childhood. Adults.

## Resumen

En España, la vigilancia medioambiental se basa principalmente en medidas de ciertos contaminantes en muestras de aire, agua, alimentos y suelos. En Madrid se ha realizado un estudio para valorar la posibilidad de poner en marcha un sistema de vigilancia de exposiciones a contaminantes ambientales en la población general utilizando biomarcadores. El proyecto ha tenido como eje el estudio del entorno de los recién nacidos. Por tanto, la población de estudio la constituyen 145 «tríos» formados por mujeres en su octavo mes de embarazo, sus parejas y los recién nacidos de dos áreas geográficas, que representan los dos principales entornos urbanos de la región, es decir, Madrid capital y su área metropolitana. Se recogieron múltiples sustratos biológicos de cada participante con el objeto de valorar las muestras más adecuadas para un sistema de vigilancia de exposiciones ambientales. Los contaminantes elegidos representan los principales agentes tóxicos a los que una población como la de Madrid está expuesta diariamente, e incluyen metales pesados, contaminantes orgánicos persistentes e hidrocarburos aromáticos policíclicos; se ha añadido también una medida inespecífica de daño citogenético, los micronúcleos en sangre periférica. Además, se han colocado muestreadores pasivos de aire en los alrededores del domicilio de los participantes. Este artículo describe en detalle el diseño del estudio y la tasa de respuesta, resume los resultados del trabajo de campo y comenta algunas enseñanzas prácticas de éste.
**Palabras clave:** Diseño de estudio. Biomonitorización ambiental. Valoración de la exposición. Infancia. Adultos.

## Introduction

The last century witnessed an important shift in the pattern of diseases worldwide. While developed countries have managed to bring about a significant reduction in incidence and mortality associated with infectious agents, chronic disorders have become the leading cause of illness, though for some of them their causes are still largely unknown at present. Modern lifestyles and industrial development have improved the quality of life but have also exposed the general population to a variety of air-, water- and food-borne toxic substances. Within the complex causal frame proposed for chronic disorders, there is increasing evidence of environmental pollutants being involved in the occurrence of these diseases[1-6].

Although ambient pollutant levels are generally low –and indeed much lower than those found in specific work environments– their health impact can nonetheless be quite relevant from a public health perspective, since exposure may affect the whole population during long periods of life. The increasing importance of environmental contaminants has led to a great number of studies targeted at improving current knowledge on pollutant exposure levels among humans and their health effects. Since the 1990s, some countries have implemented surveillance systems using biomarkers to measure exposure to different pollutants in the general population, with the National Health and Nutrition Examination Surveys (NHANES), conducted by the National Center for Health Statistics[7] in the USA, and the German Environmental Survey (GerES)[8] in Germany being considered paradigms of this type of studies.

Currently, human biomonitoring is becoming one of the main priorities of the European Union. The development of a coherent approach to biomonitoring in Europe is specifically included within the actions of the European Environment and Health Action Plan for 2004-10[9]. Both European an American Health Authorities[10,11] have stressed the importance of paying special attention to pollution levels in susceptible populations, and more specifically, in pregnant women and children. According to the US Agency for Toxic Substances and Disease Registry[12], there are critical periods of structural and functional development during both pre- and post-natal life which make children more susceptible than adults to hazardous substances, though for most pollutants these periods are still unknown. Children may also differ from adults in their capacity to repair damage from chemical insults and have a longer lifetime in which to express the deleterious consequences of such exposures.

In Spain, environmental surveillance has mainly relied on measures of selected pollutants in air, water, food and soil. In the last decade, however, several initiatives have been implemented with the aim of including human data in the framework of environmental research in different areas of the country[13-18]. One of the most recent examples of studies including biological measures is the Spanish Childhood and Environment (Infancia y Medio Ambiente [INMA]) study, a multicenter cohort project with 3100 pregnant women, designed to investigate the effect of the most important pollutants during pregnancy and the first years of life[19].

## Purpose of the study

Madrid city and its metropolitan belt constitute the biggest urban area in Spain, with similar environmental problems than other big western cities. Vallecas Health District presents some of the worst socioeconomic and health indicators within the region. In 2000 the Regional Government approved an important investment plan to promote the development of this District, which included specific Public Health objectives.

In this context, the Public Health Authority of the Madrid Autonomous Region, which had decided to promote biomarker-based assessment of exposure to specific environmental pollutants in the general population, selected Vallecas Health District as one of the pilot study areas. The primary objective of this initiative was to design a surveillance system focused on newborns and their environment. Accordingly, a study was designed to study the feasibility of implementing a biomonitoring surveillance system in the region. The aims of that exploratory research were to ascertain the population's exposure status using biomarkers; to test the selected participant enrolment strategy; to measure a wide range of pollutants, for the purpose of selecting a subgroup to be monitored in the proposed surveillance system; to obtain samples of several substrates, in order to choose those most suitable for use at a population level; and to obtain reference levels in the study areas. This paper reports the design, response rates and field work results, and provides a description of the study population.

## Study design

This is a descriptive study intended to enhance current knowledge of the pollution in the environment surrounding newborns. For that reason, it was decided that pregnant women at approximately 8 months' gestation and their partners would be included in the study population. In order to participate, subjects had to agree to answer an epidemiologic questionnaire and provide biologic specimens taken from themselves and the child,

thereby constituting so-called «mother-father-newborn triplets».

Initially, this study was set out to recruit 100 complete mother-father-newborn triplets. To fulfill this objective, however, the number of couples to be enrolled was increased by 50% (150), since there was a certain risk of losing mothers at delivery and, by extension, newborn participants.

Furthermore, we decided to include the two main types of urban environment in which most of the regional population lived. To this end, half the sample was thus recruited from the Madrid City district of Vallecas and the other half was drawn from the Greater Madrid metropolitan belt (Parla and Getafe health districts). Some basic sociodemographic features of these two areas are shown in table 1. In this paper, most data are reported for the study sample overall.

The research team presented the study to hospital managers, pediatricians, gynecologists, and midwives at public hospitals in the selected areas, as well as to the primary-care departments of local health authorities and primary-care midwives.

It was decided that women were to be invited to participate in the study by the primary-care midwives in charge of pregnancy classes. The main reasons for opting for such an approach were: a) the desire to ensure that the research project was incorporated within the institutional health framework, so that the data-extraction schedule for this study could be coordinated with the

standard follow-up of pregnant women, and b) the importance of having a person who was trusted by the women, involved in the enrollment process.

Training sessions were held at which midwives learned about the different steps of the designated fieldwork protocol and were provided with the basic material needed to fulfill the role that they were expected to play.

## Enrollment

All pregnant women in their eighth month of pregnancy, residing in the selected areas and attending childbirth preparation classes in the public health care system were invited to participate, until the designated sample size had been attained. Midwives outlined the study goals and requirements and provided informative brochures. All couples willing to participate and meeting the inclusion criteria were recruited. Both parents were required to give their prior written informed consent to participate. No financial rewards were offered, but the social utility of the study was explained.

No exclusions were made in terms of parents' or newborns' disease, race or place of origin. Inclusion criteria were: to have resided in one of the study areas for a minimum of one year; and not to have reported receiving a blood transfusion during the previous year. For

| Table 1. Sociodemographic characteristics of the two study areas, according to the 2001 census | | | | |
|---|---|---|---|---|
| | Urban district (Vallecas) | | Metropolitan district (Getafe/Parla) | |
| Income and demographic indicators | | | | |
| Income per capita (euros)[a] | 8647 | | 9317 | |
| % Homes without toilet | 1.7 | | 0.7 | |
| Total population (inhabitants) | 286374 | | 281166 | |
| % < 15 years | 14.7 | | 15 | |
| % > 64 years | 17.6 | | 9.1 | |
| % Women in fertile age range | | | | |
| (15-49 years) | 51.3 | | 57.7 | |
| Births in 2001[b] | 2667 | | 3189 | |
| | Men | Women | Men | Women |
| Education | | | | |
| Unfinished compulsory | | | | |
| education in ≥ 16 years (%) | 37.9 | 45.1 | 29.4 | 34.6 |
| University studies in ≥ 30 years (%) | 8.6 | 8.2 | 8.6 | 8.3 |
| Occupation | | | | |
| Unemployment % | 12.4 | 18.5 | 9.05 | 18.65 |
| Manual workers in ≥ 16 years (%) | 65.9 | 51 | 68 | 50 |
| Immigration from low-income countries | | | | |
| % Residents | 6.9 | 6.5 | 6.0 | 5.5 |

[a]Source: Madrid Regional Statistics Institute (Instituto de Estadística de la Comunidad de Madrid) (http://www.madrid.org/iestadis).
[b]Voters' Roll (Padrón Continuo de Habitantes) as of 1 January 2003 (http://www.madrid.org/iestadis).

**Table 2. BioMadrid study: women recruitment results**

| | Urban district | | Metropolitan districts | | Total | |
|---|---|---|---|---|---|---|
| | N.º subjects | Participation (%) | N.º subjects | Participation (%) | N.º subjects | Participation (%) |
| Meeting inclusion criteria | 273 | 100 | 242 | 100 | 515 | 100 |
| Initial agreement | 147 | 54 | 121 | 50 | 268 | 52 |
| Enrolled (with both parents' agreement) | 74 | 27 | 75 | 31 | 149 | 29 |

logistic reasons, we also required women to be aged over 15 years, had single pregnancies, and intended to deliver their babies at the local public hospital.

After a small pilot trial, recruitment started in October 2003 and lasted until May 2004. In all, 630 women in 35 sessions of delivery-preparation courses were informed about the project and invited to participate. Of those eligible, 52% stated that they were willing to take part in the study and invite their partners to collaborate. Finally, 149 couples (29%) were enrolled (table 2). Two of these women gave birth before the first scheduled date. Additionally, two couples and one father failed to attend any appointment. All of them declined any subsequent call from the research group and withdrew from the study at an early stage.

Approximately 70% of the pregnant women attending the labor classes completed a brief questionnaire, designed to collect basic sociodemographic data so as to enable the representativeness of the final sample to be evaluated. The characteristics of participants and non-participants who completed this form are shown in table 3.

**Field work organization and sample collection**

Table 4 summarizes the schedule for the field work in which parents were intended to play an active role. At enrolment, midwives assigned an identification number to the future triplet, and presented them with a small

**Table 3. Characteristics of participants and non-participants who answered the initial questionnaire, the BioMadrid study (November 2003-March 2004)**

| | Participants | | Non-participants | | |
|---|---|---|---|---|---|
| | n (134) | % | n (211) | % | p |
| Country of birth | | | | | |
| Spain | 114 | 86 | 193 | 91 | 0.133[b] |
| Parity | | | | | |
| First child | 87 | 66 | 149 | 71 | 0.294[b] |
| Age | | | | | |
| Mean | 30.5 | | 29.9 | | 0.252[c] |
| Education | | | | | |
| Primary/secondary | 32 | 24 | 79 | 38 | 0.005[d] |
| Vocational training/High School Certificate | 56 | 43 | 90 | 43 | |
| University graduate/Postgraduate | 43 | 33 | 40 | 19 | |
| Occupation[a] | | | | | |
| Clerical/Bookkeeping | 33 | 25 | 38 | 18 | 0.004[d] |
| Housewife | 14 | 11 | 36 | 17 | |
| Sales clerk/shop assistant | 16 | 12 | 33 | 16 | |
| Health-related | 12 | 9 | 14 | 7 | |
| Education-related | 17 | 13 | 7 | 4 | |
| Waitress/cook | 7 | 5 | 12 | 6 | |
| Hairdresser | 7 | 5 | 8 | 4 | |
| Cleaner/home worker | 1 | 1 | 12 | 6 | |
| Other | 25 | 19 | 51 | 24 | |

[a]Occupations with 5 or more participants.
[b]Two-sample test of proportions.
[c]Two sample t tests on the equality of means.
[d]Chi-square test.

**Table 4. BioMadrid field-work schedule: data collected, substrates, and pollutants**

| | Study entry | | | | | At delivery | | | | Three weeks after delivery | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Biologic samples | | | | | Biologic samples | | | Biologic samples | |
| | Questionnaires | Environmental measures (home external air) | Substrates | Pollutants | Other analysis | Clinical exam/ Questionnaire | Substrates | Pollutants | Other analysis | Questionnaire | Substrates | Pollutants |
| Father | General Diet | VOCs Nitrogen oxides | Blood | Pb, Cd, Hg As Pesticides PCBs Dioxins and furans | Micronuclei | | | | | | | |
| | | | Urine | Cd 1-OH-pyrene | | | | | | | | |
| Mother | General Diet Pregnancy | VOCs Nitrogen oxides | Blood | Pb, Cd, Hg As Pesticides PCBs Dioxins and furans | Micronuclei | Delivery data | Placenta | Pb, Cd, Hg As Pesticides PCBs Endocrine disruptors | | Lactation | Breast milk | Pb, Cd, Hg As Pesticides PCBs Dioxins and furans |
| | | | Urine | Cadmium 1-OH-pyrene | | | | | | | | |
| Newborn | | | | | | Anthropometric data Sexual maturation | Cord blood | Pb, Cd, Hg As Pesticides PCBs | Micronuclei | Hair | Hg Methyl-Hg |

VOCs: volatile organic compounds, including benzene, toluene, ethylbenzene, m+p-xylene and o-xylene; PCBs: polychlorinated biphenyls.

bag. It contained general information about the project, an instruction leaflet, and the logistic material for the study, including a breast-milk pump to collect milk samples, which was also handed out as a gift. All the material in the bag was pre-labeled with the trio number plus an identification character that would allow each of its members to be distinguished («M» [madre] for mothers, «P» [padre] for fathers, and «H» [hijo/a] for children). The necessary material for collecting biologic specimens was suitably labeled and wrapped in three packs, one for each member of the triplet.

### Phase 1: study entry

Midwives scheduled a date for both parents to collect blood and urine specimens, coinciding with the programmed control of the pregnant women at approximately 38 weeks of pregnancy. By appointment, trained personnel interviewed both parents at their home to complete the epidemiologic questionnaires.

### Phase 2: delivery room

At delivery, mothers informed the hospital that they were BioMadrid participants, and provided the newborn pack included in the kit, with the blood tubes for cord blood and the labels to identify the placenta container, previously supplied to the hospital by the research team. Midwives attending the birth collected the required biologic specimens and registered basic information on labor. Placenta was collected and frozen whole. During hospitalization, pediatricians performed a clinical examination of the newborns and collected data on clinical, anthropometric and sexual development at birth.

### Phase 3: at home

Three weeks after delivery, women were contacted by telephone at home to answer a brief breastfeeding questionnaire and to arrange for the breast milk and newborn hair specimens to be picked up. Mothers had previously received written instructions on how to collect these specimens properly, by using the breast pump provided and cutting a lock of their children's hair.

### Biologic sample processing

All specimens were collected in accordance with specific protocols, in containers labeled with the subject identification number and date. In brief, five different types of specimens were collected (table 4). Adult blood speci-

mens (30 ml) and cord blood (12.5 ml) were taken, using the vacutainer system with different tubes, namely: heparine tubes for heavy metal and micronuclei analysis; tubes containing gelose for routine biochemical analyses; tubes with EDTA for routine hemogram; and empty, simple glass standard tubes for organochlorines and dioxins. The recipient laboratory identified the specimens with the case codes, performed the systematic hemogram and biochemical determinations, extracted the serum and then sent the heparinized tubes to the Genetic Department of the Ramón y Cajal Hospital for micronuclei analysis, and all remaining specimens to the Madrid Regional Public Health Laboratory (RPHL), where they were centralized, classified, frozen and stored at –70 ºC.

Urine specimens (100 ml) corresponded to the first morning urine passed on the date appointed for blood extraction. Receptacles had to be wrapped in aluminum foil to avoid light exposure, and were kept refrigerated at 2-8 ºC by the recipient laboratory until submission to the RPHL.

Placentas were frozen at –20 °C immediately after birth at the maternity ward.

Milk samples (20 ml) were collected by mothers at the end of third week post-delivery, after having fed their babies. Milk receptacles were kept refrigerated (2-8 ºC) until collected by study personnel, and frozen and stored at the RPHL. Three weeks postpartum and after the baby had been bathed, a lock of newborn hair was cut with scissors by the parents and kept at room temperature until analysis.

## Environmental measures

As part of this research project, passive air samplers were positioned around study participants' place of residence, in order to take outdoor measurements of air pollutants. The aim was to ascertain exposure to nitrogen dioxide and volatile organic compounds (benzene, toluene, ethylbenzene, m+p-Xylene and o-xylene) in the two study areas. Passive air samplers, analytical determinations and methodology were in accordance with the INMA protocols[20], though in our case a single sampling campaign was completed. A total of 80 passive air samplers were deployed, 29 in the urban area (Vallecas district) and 51 in the outlying area (Getafe and Parla districts). These measures may help to have a better knowledge of the study areas pollution profile.

## Questionnaires

Questionnaires were used in all three phases of the field work. The design of the principal epidemiologic ques-

tionnaire, completed at study entry, took the INMA protocols into account so as to allow for future comparisons with INMA results. Sex-specific versions were initially tested on a small sample of mothers and fathers in each area, and some questions were reworded in line with the results of this trial. The interview lasted around 40 minutes and included questionnaires with several modules: sociodemographic information; tobacco exposure (lifelong use, recent and current smoking, and secondhand exposure); occupational history; socioeconomic data; environmental exposures at home; and, reproductive and general medical history (diseases, drug consumption, radiological exposure, and dental fillings). For women, an additional module registered characteristics and clinical events during the pregnancy. Dietary information was recorded, using a previously developed and validated food-frequency questionnaire[21-23], which enables nutrient intake to be estimated.

At the hospital, health staff completed a sheet recording delivery and newborn health status and anthropometric data. Finally, a lactation questionnaire registered information on the lactation process and sample collection.

## Ethical considerations

Both the study protocol and informed consent form complied with the principles of the Helsinki Declaration and were formally approved by the Ethical Committee of the Carlos III Institute of Health. As children were involved in this project, the final version of the protocol was also submitted to the official Children's Ombudsman for the Madrid Autonomous Region. Personal data and biologic samples were stored in accordance with prevailing Spanish legal and ethical requirements. Confidentiality was guaranteed. Participants were provided with a contact telephone number in the event that they might have any doubts or queries, or wish to withdraw their consent to participate at any time.

## Field work results

Table 5 summarizes the main results. Of the originally planned 150 triplets, we finally recruited 145 mothers, 144 fathers and 135 newborns. It has to be taken into account that blood cord collection, which took place at approximately one month after initial parents' sample collection, had additional difficulties as it required that: a) mothers provide the material included in the kit to the hospital personnel, and inform that they were BioMadrid participants, and b) delivery circumstances permit the normal collection of samples.

**Table 5. BioMadrid study: field work results**

|  | Total | | Fathers | | Mothers | | Newborns | |
|---|---|---|---|---|---|---|---|---|
|  | n | (%) | n | (%) | n | (%) | n | (%) |
| Total | 424 | | 144 | | 145 | | 135 | |
| Questionnaires | | | | | | | | |
| General | 278 | (96) | 139 | (97) | 139 | (96) | - | - |
| Diet | 265 | (92) | 132 | (92) | 133 | (92) | - | - |
| Anthropometric data | 114 | (84) | - | - | - | - | 114 | (84) |
| Sexual maturation | 114 | (84) | - | - | - | - | 114 | (84) |
| Lactation | 121 | (83) | - | - | 121 | (83) | - | - |
| Biologic samples | | | | | | | | |
| Blood | 399 | (94) | 140 | (97) | 142 | (98) | 117 | (87) |
| Urine | 282 | (98) | 140 | (97) | 142 | (98) | - | - |
| Placenta | 121 | (83) | - | - | 121 | (83) | - | - |
| Breast milk | 107 | (74) | - | - | 107 | (74) | | |
| Newborn hair | 130 | (96) | - | - | - | - | 130 | (96) |

Among adults, blood and urine specimens were obtained in over 97% of subjects, the general questionnaire attained similar figures, and dietary record sheets were completed in 92% of cases.

In newborns, the most frequently obtained specimen was hair (96%) followed by cord blood (87%) (table 5). In one case lost to follow up was due to miscarriage. Other nine children with no biological samples were born alive, though were lost due to different reasons: one woman forgot to take the kit including instructions and material needed to collect the biological samples; four mothers were sent to a non-participant hospital to give birth due to overcrowded maternal wards in the reference centers, and in four other cases complications in delivery distorted the logistical arranged procedures.

The lowest figures corresponded to placentas (81%) and milk samples (74%). Fourteen of the lactating mothers answered the lactation questionnaire but failed to collect a breast milk specimen or provided an insufficient volume of milk. In general, participants reported that the instructions supplied were clearly understandable. All BioMadrid protocols are available upon request.

## Discussion

This project is the first comprehensive study of pollutant levels in the population of the Madrid Region. It will provide an estimate of environmental exposure to different pollutants in several biologic specimens from fathers, mothers and newborns in the study areas, and will help design a feasible environmental surveillance system based on human biomarkers. In spite of the study's complex design, field work results can be considered as satisfactory.

*Why triplets? Three populations at hand*

After considering different strategies, it was decided that the target population of the study were to be triplets of mothers, fathers and newborns. This design enables environmental exposures to be simultaneously assessed in adults and newborns. The short follow-up included in the study rendered it possible for data about the whole perinatal period to be obtained. Fathers are not usually included in these types of studies, yet they nevertheless represent half the adult population and an important part of the environment in which children will be raised.

Our aim was to obtain a representative sample of the general population. The decision to study women who intended to give birth in a public hospital implied the exclusion of a proportion of couples with private insurance and, in all likelihood, higher income. Yet, there are several factors that probably served to lessen this bias: *a)* the universal coverage of the public health system in Spain; *b)* the good image of the recently-opened public maternity ward in the urban study area (Vallecas District), which even attracted people from other health districts, and *c)* the lack of private hospitals in the Madrid metropolitan area. Additionally, account must be taken of the fact that the design selected fertile couples in the third trimester of pregnancy, which means that, if a contaminant affects fertility or shortens pregnancy length, the concentration in our adult sample might underestimate its levels in the general population.

*Participation*

This study was organized by Public Health Authorities, which wanted to incorporate the project within the

public health care organization. Thus midwives, who were not part of the research team, were in charge of enrolment. Differences of involvement of midwives might have partially influenced recruitment rates by centres, though the research team maintained repeated personal contacts in order to encourage them. Even if it seems obvious, it is important to insist on the fact that recruitment is one of the critical points in this kind of studies and perhaps additionally efforts to explain the research and to motivate candidates might improve participation rates.

One might wonder if recruitment rates could have been higher, had we hired specific personnel for it. However, population studies which require active participation generally have low participation rates. In our case, the rate of women's initial agreement to participate (52%) was similar to those described in other studies of pregnant women in Spain (54%)[24] and lower than that of the general population of Western Germany (63%)[8]. However, our design entailed the need for agreement by a second population, i.e., the fathers, whose rate of consent to participate was similar. Consequently, overall agreement for this study decreased to 29%. In contrast, participants were highly motivated and played an active role in the logistics of the project, since they kept most of the specimen-collection material at home and were responsible for taking it to the extraction points and the maternity ward.

Our results showed that women who refused were similar to those who agreed to participate in this study in terms of age and parity, and proportion of non-Spanish subjects. Our sample might perhaps overrepresent people with healthier lifestyles and, probably, with a slightly higher socioeconomic status, since a higher educational level was observed for participants versus non-participants. Among mothers who took part, education-related and, to a lesser extent, health-related jobs were more frequent than cleaning-related occupations and housewives.

*Biologic substrates and measures*

One of the study objectives was to assess which biologic substrates might be more suitable for an environmental surveillance system. Owing to budget constraints, we had to find an equilibrium between sample size and the number of substrates and pollutants to be studied. We wished to test: *a)* the acceptability of each biological sample; *b)* logistic aspects linked to specimen-collection, -processing and -storage, and *c)* informative output, i.e., number and type of pollutants that can be effectively measured and the time frame that these represent. With respect to the toxicants to be studied, we selected the main agents to which a population like of Madrid is thought to be exposed daily, including cer-tain heavy metals, persistent organic pollutants (POPs), and polycyclic aromatic hydrocarbons (PAH). Finally, micronuclei, a commonly used unspecific index of cytogenetic damage[25], was incorporated to provide a biologic response marker of exposure to genotoxic agents.

*Some initial lessons learned*

Though blood is the most versatile substrate, it requires trained personnel, and there is an ethical limit to the amount of specimen that can be collected. Thus, for some of the pollutants, e.g., dioxins, blood from several participants had to be pooled in order to achieve the size needed for analysis. Some types of bio specimens, such as placenta and cord blood, were easier to obtain than had been supposed, since maternity ward staff are used to collecting these for other purposes. Moreover, in the case of placenta, it is a large organ that can be easily collected. In contrast, most newborn hair samples were regarded by the laboratory as being insufficient (less than 5 mg) for the purpose of obtaining accurate measurements. Additional non-invasive substrates that could have been included in the study protocol were nails and meconium. Repeated milk specimens, also easy to collect, could have been taken in order to store part for future purposes, though in that case telephone calls to remind subjects of specimen collection should be scheduled to avoid any possible oversights. Insofar as the selected biomarkers are concerned, cotinine could have been as well considered.

## Conclusions

This project has enabled the complexity of implementing a surveillance program using biologic samples to be estimated. In the next future, we have still several questions to answer when laboratory analyses are completed, including the discussion about the most suitable toxicants and biological samples to be considered in a surveillance system. Nevertheless, the financial and human effort needed to put a system like this into operation calls for firm political backing. The results of this study will serve to tailor the design of any future surveillance system to the reality of the Madrid Region.

## Bibliografía

1. Bhatnagar A. Environmental cardiology: studying mechanistic links between pollution and heart disease. Circ Res. 2006; 99:692-705.
2. Montesano R, Hall J. Environmental causes of human cancers. Eur J Cancer. 2001;37 Suppl 8:67-87.
3. Peto J. Cancer epidemiology in the last century and the next decade. Nature. 2001;411:390-5.
4. Samet JM. Controlling the avoidable causes of cancer: needs and opportunities for etiologic research. Environ Health Perspect. 1995;103 Suppl 8:307-11.
5. Navas-Acien A, Guallar E, Silbergeld EK, Rothenberg SJ. Lead exposure and cardiovascular disease: a systematic review. Environ Health Perspect. 2007;115:472-82.
6. Boffetta P. Human cancer from environmental pollutants: the epidemiological evidence. Mutat Res. 2006;608:157-62.
7. NCHS. National Health and Nutrition Examination Survey. National Center for Health Statistics [update 2007; accessed 22 Mar 2007]. Avalaible in: http://www.cdc.gov/nchs/nhanes.htm
8. Seifert B, Becker K, Hoffmann K, Krause C, Schulz C. The German Environmental Survey 1990/1992 (GerES II): a representative population study. J Expo Anal Environ Epidemiol. 2000;10:103-14.
9. Commission of the European Communities. The European Environment & Health Action Plan 2004-2010. COM(2004) 416 final. Vol I. 6 February 2004. Brussels. Available in: http://www.eu-humanbiomonitoring.org/doc/actionplan.pdf
10. European Commission. European Environment & Health. Human Biomonitoring [update 21 Feb 2007; accessed 7 Jun 2007]. Avalaible in: http://ec.europa.eu/environment/health/biomonitoring.htm
11. CDC. Department of health and human services. National Biomonitoring Program [update Dec 2005; accessed 7 Jun 2007]. Avalaible in: http://www.cdc.gov/biomonitoring/default.htm
12. ATSDR. Agency for Toxic Substances and Disease Registry. Toxicological profiles [update May 2007; accessed 7 Jun 2007]. Avalaible in: http://www.atsdr.cdc.gov/toxpro2.html
13. Ribas-Fito N, Sunyer J, Sala M, Grimalt JO. Cambios en las concentraciones de compuestos organoclorados en las mujeres de Flix, Tarragona. Gac Sanit. 2003;17:309-11.
14. Luzardo OP, Goethals M, Zumbado M, Álvarez-León EE, Cabrera F, Serra-Majem L et al. Increasing serum levels of non-DDT-derivative organochlorine pesticides in the younger population of the Canary Islands (Spain). Sci Total Environ. 2006; 367:129-38.
15. Botella B, Crespo J, Rivas A, Cerrillo I, Olea-Serrano MF, Olea N. Exposure of women to organochlorine pesticides in Southern Spain. Environ Res. 2004;96:34-40.
16. López-Espinosa MJ, López-Navarrete E, Rivas A, Fernández MF, Nogueras M, Campoy C et al. Organochlorine pesticide exposure in children living in southern Spain. Environ Res. 2008;106:1-6.
17. González CA, Kogevinas M, Huici A, Gadea E, Ladona M, Bosch A, et al. Blood levels of polychlorinated dibenzodioxins, polychlorinated dibenzofurans and polychlorinated biphenyls in the general population of a Spanish Mediterranean city. Chemosphere. 1998;36:419-26.
18. Sala M, Sunyer J, Otero R, Santiago-Silva M, Camps C, Grimalt J. Organochlorine in the serum of inhabitants living near an electrochemical factory. Occup Environ Med. 1999;56:152-8.
19. Ribas-Fito N, Ramón R, Ballester F, Grimalt J, Marco A, Olea N, et al. Child health and the environment: the INMA Spanish Study. Paediatr Perinat Epidemiol. 2006;20:403-10.
20. Esplugues A, Fernández-Patier R, Aguilera I, Iñíguez C, García Dos SS, Aguirre AA, et al. Exposición a contaminantes atmosféricos durante el embarazo y desarrollo prenatal y neonatal: protocolo de investigación en el proyecto INMA. Gac Sanit. 2007;21:162-71.
21. Vioque J, González L. Validity of a food frequency questionnaire (preliminary results). Eur J Cancer Prev. 1991;Suppl 1:19-20.
22. Vioque J, Guillén M, García M, González L, Vicente MC, Quiles J. Reproducibility and validity of a food frequency questionnaire among men and women of Alicante, Spain. Epidemiology for sustainable health. Abstract book (Vol II). XV International Scientific Meeting of the International Epidemiological Association. Firenza: IEA; 1999. p. 295.
23. Vioque J. Validez de la evaluación de la ingesta dietética. In: Serra LL, Aranceta J, editors. Nutrición y Salud Pública. Métodos, bases científicas y aplicaciones. Barcelona: Masson; 2006. p. 199-207.
24. Rodríguez P, Romero-Aliaga E, Pérez Aliaga S, Quiles A, Ramón R, Rebagliato M, et al. Análisis descriptivo de la participación y comparación de las inclusiones y rechazos en la cohorte de embarazo INMA-Valencia. Gac Sanit. 2006;20:47.
25. Vine MF. Micronuclei. In: Hulka BS, Wilcosky TC, Griffith JD, editors. Biological markers in epidemiology. New York: Oxford University Press; 1990.