



Review

# Children's exposure to environmental pollutants and biomarkers of genetic damage

## I. Overview and critical issues

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### Abstract

In the last decade, molecular epidemiological studies have provided new perspectives on studying environmental risks in pediatric populations, based on the growing understanding that children may be more susceptible to toxicants than adults. Protecting children's health is a social priority, and specific research programs have been initiated with this purpose in the United States and Europe. These programs address the development of (i) less invasive methods for biological specimens collection, (ii) specific tools for interpretation and validation of biomarkers, (iii) methods for translating biomarker results into intervention strategies and for integrating them with environmental monitoring and health data, (iv) optimal ways to obtain consent and provide information to children and/or their parents participating in the studies and (v) techniques for the effective communication with policy makers and the public. Critical issues in children's environmental research discussed in this paper include specific needs of study design, exposure assessment, sample collection and ethics. Special consideration is given to the autonomy of the child in giving consent, the details and nature of the information provided, and the need to warrant controlled access to sensitive information. The use of incentives such as gifts and payment to ensure the participation of school-aged children is specifically discussed. Examples of field studies that are focused on the effects of pesticides, air pollution and formaldehyde are used to illustrate advantages and limitations of biomarker studies in children.

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## 1. Introduction

Protecting children's health is a social priority. Specific programs have been initiated in the United States and Europe in recognition of this fact [1–3].

In the year 2000, the United States Congress passed the Children's Health Act [4]. The National Institute of Environmental Health Sciences (NIEHS) and the United States Environmental Protection Agency (US EPA) funded eight Centers for Excellence in Children's Environmental Health Research in 1998, and funded four more in 2001. The Centers are required to conduct community-based research on the causes and mechanisms of children's disorders with environmental etiologies. Currently, a major National Children's Study is being planned by NIEHS, EPA and the center for disease control (CDC) to follow the growth and development of approximately 100,000 children and evaluate environmental impacts on their health [5,6].

In 2003, the European Commission launched the SCALE program, that stands for Scientific evidence, focused on Children, to raise Awareness, improve the situation by use of Legal instruments and ensure a continual Evaluation of the progress made [3]. SCALE is aimed at assessing and minimizing the adverse health effects of environmental pollution on children. The main goals of the SCALE program are development of a common European information system and adequate political measures to improve children's environmental safety. SCALE focuses on

environmental health indicators, priority diseases (i.e., respiratory diseases, neurodevelopment and childhood cancer), integrated monitoring and children's research needs.

A partial overview of the European research in children's health has identified approximately 100 studies with over 400,000 children participating in existing biomonitoring and or research activities conducted in the member States and acceding Countries [3]. Forty-four studies dealt with exposure to heavy metals, 15 with dioxins/PCB and 5 with exposure to endocrine disruptors. Twenty-seven studies included the identification of biomarkers of asthma and allergy, but only a limited number of them investigated cytogenetic biomarkers in relation to environmental pollution. The methodological differences detected between the studies warranted the need for harmonization programs aimed at (i) generating a broader data set regarding exposure and its relationship to health outcomes, (ii) improving the chance of detecting spatial and temporal trends, (iii) allowing comparisons between geographical areas, (iv) quantifying the contribution of different environmental compartments (e.g., air, water, food, etc.) and (v) identifying emission sources to provide policy makers with better information for the planning of sound environmental actions and the implementation of control measures.

The leading priorities to be addressed in such an action program include the development of non-invasive methods for biological specimen collection,

the way of providing information to and obtaining consent from study children (or their parents/guardians), the development of specific tools allowing for the interpretation and validation of measurements with biomarkers, the translation of biomarker results into intervention strategies and integration with environmental monitoring and health data, and the effective communication with policy makers and the general public [4].

In 2001, the *European Network on children's susceptibility and exposure to environmental genotoxigents* (CHILDRENGENONETWORK) [7] was planned as a concerted effort focusing on the effects of environmental exposure to genotoxic agents during various stages of childhood. Recommendations concerning the need for new research projects as well as ethical, legal and social aspects of biomonitoring in children will be delivered by the end of 2005 to the European Commission and are expected to positively influence European research in the field.

The purpose of this article is to review main issues in studies of genetic damage in children. Several examples of biomarker studies in children from Europe and the United States are used to illustrate the challenges faced by investigators conducting such studies, and the lessons learned.

## 2. Exposure assessment in children

The recent interest in studying children's exposure to environmental toxicants stems from the new understanding that pediatric populations may be more susceptible to these agents, including carcinogens [8–10]. Because of vast differences in behavior and physiology, children's exposure patterns are quite different from those of adults. Children have higher daily intakes (per kilogram of body weight) of food, water and air than adults, and therefore, may have a disproportionately higher intake of toxic agents [11]. Children's exposure to environmental pollutants is also increased because of their "hand to mouth" behavior, which gives them a higher chance of ingesting toxic compounds present in water, soil, and house dust [12,13]. Finally, children may differ from adults in rates of detoxification, DNA repair processes, and cell proliferation [14]. Therefore, children and adults living in the same environment may experience very different

exposures. As a result of the physiological and behavioral distinctions, and because of the variability in exposures that they may experience, specific approaches to exposure assessment in children are recommended [10,15].

Exposure assessment in children presents a number of challenges to investigators. Exposure to environmental toxins may occur by various routes (via inhalation, ingestion or dermal absorption) and may begin during the prenatal period and last through all stages of postnatal development. It was established that in utero exposure may be especially important because it may affect susceptibility in the later stages of life [16]. Ideally, exposure assessment should be based on combined questionnaire and marker data characterizing external and internal exposure. In reality, research data are often limited to either internal or external measures and only to a specific route of exposure. External markers can be measured in the air, dust, water, etc. Internal biomarkers relevant to children include measurements in blood, urine, saliva, breast milk, meconium and other biological samples. According to the EPA 2003 Report on "America's Children and Environment", five important media were identified: outdoor air, indoor air, drinking water, food and soil [2]. The most relevant pollutants for the majority of children are lead, mercury and environmental tobacco smoke as measured by its biomarker cotinine. Additionally, pesticides, air pollution and arsenic in drinking water are common exposures in some developing countries. Most of these pollutants are genotoxic depending on the level and the length of exposure and their effect may be modified by physiological and behavioural features. For example, Wessels et al. provided evidence that the factors increasing susceptibility of children to pesticides (as well as many other environmental genotoxigents) include their higher body surface/body weight ratio, higher circulatory flow rates, greater intake of water, milk and fruit juice than adults, consumption of large quantities of fresh foods (possibly contaminated), and frequency of hand-to-mouth activities [17]. Further, children undergo rapid development of their nervous and other systems, and therefore may be particularly affected by altered levels of enzymes that modify the toxicity of chemicals [16,18]. In general, it has been shown that children may be susceptible to very low levels of exposure, in some cases below the detection limits of current methods.

When considering children's exposures, a regional approach has proved productive. Regional approach focuses on priorities of monitoring exposures and health effects in specific parts of the world. Recently, Suk et al. published reviews of environmental threats in South Asia, the Western Pacific [19] and the Arctic regions [20]. They showed that regional approach helps to identify specific exposures, and develop monitoring plans and intervention and remediation strategies for this and other regions of the world. While in the last 15–20 years, general lead levels in children's blood significantly decreased in the USA and many other developing countries that banned leaded gasoline, lead is still a very critical problem in many parts of the world including China, the Philippines and other countries in South Asia and Africa, where gasoline is still leaded [21]. Children may be exposed to lead not only because of traffic or because they live in close proximity to the areas where their parents work, but also through less obvious routes, like the high lead level contents in traditional ceramics used for cooking (e.g., Mexico) [22,23]. Additionally, not only regions of the world but also regions of the countries or even cities may have an elevated risk. For example, because of the high exposure to traffic exhausts and residence in old housing where lead based paint has not been removed, lead levels remain high in some segments of the American population, like the residents in inner cities [24].

Other important factors to be considered in the design of environmental studies in children are dietary intake, physical activity and body mass index. For example, childhood asthma may be affected by dietary habits and the increased body mass that results from a sedentary lifestyle [24]. These factors have to be included while exploring the role of other known risk factors of asthma in children. Further, ethnicity and country of birth may modify the impact of a known risk factor. In the United States, Mexican Americans have the lowest risk of asthma among Hispanics. However, the prevalence of asthma was higher in US-born than Mexico-born Mexican Americans [25].

Major open questions in children's exposure assessment, such as dose–response relationships, susceptibility to low levels of exposure, or the effect of mixtures may be effectively addressed only by comprehensive studies of children's exposure. These entail large study groups and long-period follow-up.

### 3. Susceptibility to DNA damaging agents

The presence of a causal association between exposure to genotoxic agents during developmental stages of life and increased risk of cancer is suggested by findings from experimental and epidemiological studies [26,27]. Recent trends in childhood cancers in the USA and Europe seem to confirm children's increased exposures to genotoxicants [28,29]. Human studies have reported increased risks for the clear cell cancer of the vagina, cervix, and breast cancer in young women who were exposed to diethylstilbestrol in utero [30,31]. Radiation-induced breast cancer, leukemia and thyroid cancer have been detected in subjects exposed to ionizing radiation during adolescence [32–34]. It has been reported that children exposed to low frequency electromagnetic fields may have an increased risk for leukemia [35]. Higher incidence of leukemia has also been reported in children exposed to certain traffic pollutants [36,37]. The occurrence of non-Hodgkin's lymphoma in children has been linked to pesticide exposure [38]. Animal studies conducted with exposures during fetal development revealed higher tumor yield and lower latency compared to postnatal challenge, particularly when the exposure continues through adulthood [39].

Despite increasing evidence in the scientific literature linking exposure to environmental toxicants with childhood diseases, more studies are needed to establish which developmental windows or "age-related factors" are relevant in cancer susceptibility [40–43].

Molecular epidemiological studies have provided new perspectives in studying environmental risks in pediatric populations and are among the most promising approaches to understanding environmental risks. Field studies employing biomarkers allow for exploration of the various mechanisms along the pathway from an exposure to corresponding health effects. Biomarkers of susceptibility are also able to provide insight into the role of genetic factors in the health outcomes caused by environmental exposures [27,44].

The genotoxic damage induced by exposure to environmental pollutants shows a large inter-individual variability in children [45]. Among other factors, heterogeneity of genetic profiles may play a significant role in determining the observed variability, affecting the statistical power of the study and hampering the assessment of the exposure-related

effect(s). The most commonly investigated genetic feature is the presence of inherited polymorphic variations (genetic polymorphisms) in genes involved in the metabolism of chemicals or in the process of DNA repair. The role of metabolic polymorphisms as modifiers of the effect of exposure to genotoxic agents on DNA damage has been evaluated for several biomarkers, though the event most widely studied is the formation of DNA adducts. In a group of 160 Polish mothers exposed to airborne PAH and their newborns, a significantly higher level of DNA adducts was found in mothers and children with a defective cytochrome P450 (*CYP*) *IA1* gene (individuals heterozygous or homozygous for the *CYP1A1 MspI* restriction site) [44,46]. An increased frequency of DNA adducts was also found in those with the glutathione S transferase (*GST*) *M1 null* genotype, and the combination of the two unfavorable genotypes resulted in a synergistic effect [47]. The frequency of DNA adducts in placentas of pregnant women living in the heavily polluted area of Teplice in the Czech Republic was significantly increased in individuals with the *GSTM1 null* genotype, while no association was found for women with the *N*-acetyl transferase (*NAT-2*) genotype [48,49]. Similar findings were described by Lagueux et al., who found an association between DNA adducts in the placenta and the *CYP1A1*-dependent enzyme activity in pregnant women exposed to organochlorines in two remote coastal regions in Quebec, Canada [50]. The evidence for the role of metabolic polymorphic genes on biomarkers other than DNA adducts in children is rather limited. Among the few studies addressing this issue, a small study performed in 26 newborns reported an association of *CYP1A1 MspI* polymorphism with the frequency of chromosomal translocations [51]. Recent results on genetic susceptibility of children to diseases modulated by *BRCA2/FANCD1*, have suggested that studying functional impairment of highly penetrant genes may provide important data for evaluating the role of individual susceptibility in childhood diseases [52].

Although the available evidence regarding the role of genetically based metabolic variability in modulating genotoxic damage remains restricted, differences between adults and children routinely suggest that children may be more susceptible to environmental compounds which interact with polymorphic genes [53–55].

#### 4. Epidemiological study design in children

Biomonitoring studies designed to evaluate genetic damage in children require careful evaluation of specific features that characterize pediatric populations. These may offer advantages and disadvantages for the investigators. The most evident difference between children and adults is the reduced impact of traditional confounding factors like cigarette smoking and occupational exposures. Children, at least until adolescence, usually do not smoke and are not exposed to any occupational genotoxic agents. However, they may experience second hand smoke (ETS) and have a higher chance of ingestion of contaminated dust [10]. Further, because a child's diet is less variable than an adult's, its impact as a confounder may either be reduced or increased if some part of the diet is contaminated with genotoxic agents [56].

An advantage of studying pediatric populations is the relative ease of tracing study subjects during school age. On the other hand, there are a number of aspects that may limit the suitability of children as a population for biomonitoring studies. The first is the low level of genetic damage in children, which generally reduces the statistical power of the study and requires a large sample size [45]. Pediatric populations are also characterized by a higher frequency of infectious diseases than adults. This fact must be addressed within the study designs given the genotoxic potential of viruses and vaccines [57,58].

Further, whenever the age-range of the study group encompasses teenagers, the possible role of individual response to hormonal fluctuations must be taken into account. Besides epidemiological parameters, ethical considerations must be carefully addressed in any study involving children. For example, the need to avoid traumatic and painful sampling procedures that can cause distress in children has led scientists in the field to develop non-invasive procedures for obtaining DNA or other pediatric biological specimens.

#### 5. Biological sampling in children

Biological samples needed for exposure assessment and evaluation of early biological outcomes can be obtained from many tissues and by a number of non-invasive as well as invasive methods (Table 1).

Table 1  
Examples of invasive and non-invasive sampling in biomarkers studies in children

Type of sample	Mode of collection
Non-invasive	
Buccal epithelia	Swab of inner lining of cheek with tongue depressor or cytobrush
Saliva	Sterile plastic pipette or specially prepared cotton swab
Urine and urothelial cells	Collected in sterile container, separated by centrifugation
Nasal epithelia	Swab of inner lining of the nose with cytobrush or cotton swab
Cord blood	Drained into sterile container from the cord after delivery
Placenta	Collected after delivery
Expired air	Spirometer attachment
Hair	In container after cut or fallen out
Finger nails	Clippings in sterile container
Extracted teeth	Collected in sterile container after loss
Invasive	
Blood	Venipuncture or finger prick
Bronchial, esophageal, GI tract epithelia	Biopsy material
Bone marrow cells	Crista biopsy or sternal puncture
Spinal fluid	Spinal tap
Amniotic fluid	Amniocentesis (mother)
Adipose tissue	Biopsy

The choice of biological specimens and mode of collection depends on the type of biological assay to be performed, the age of the study population(s), and the subjects' health status. Exfoliated cells from the mouth (buccal) or urine (urothelial), may be adequate for some research purposes (e.g., genotyping, cytogenetic damage), and can be obtained through much less invasive methods than blood collection (which is often done via venipuncture). However, unlike blood cells, exfoliated cells are not easily grown in culture, thus limiting the possibility of obtaining metaphase cells for cytogenetic analysis. Buccal cells are commonly collected with a small cyto-brush or tongue depressor, which is then rinsed in conical centrifuge tubes containing stabilizing buffer [59]. Use of commercial mouthwash and simple mouth rinse [60–62] has recently gained popularity. Buccal cells can also be collected on pre-treated cards [63,64]. Exfoliated cells are easier to collect than blood and do not require highly trained personnel such as a pediatric phlebotomist. Buccal cells can be collected from remote field sites and transported without refrigeration to central laboratories for processing without compromising sample integrity [60]. Collection of exfoliated cells can minimize the use of valuable blood samples, reduce the blood volume needed from each study subject, and increase the sample size of the study population because participants may be more

willing to provide a buccal swab or urine sample than donate blood [58,65,66].

## 6. Ethical issues

There are several reasons that justify conducting field studies in children, including needs to improve our knowledge of environmental risks at different stages of development, and to define baseline levels of genetic damage in children. However, despite scientific and public interest in these topics, none can provide immediate benefit to the study participants. In light of this fact, ethical considerations have led scientists to limit field studies on children to those that do not expose the child to unnecessary risks, and to research where data on children is more informative than research carried out on adults.

Balancing the need to establish standards for paediatric populations against the responsibility of not causing harm to children was considered in the Guidelines prepared by the Council of International Organizations of Medical Sciences in collaboration with the World Health Organization. The Guidelines state: "*The risk of interventions that are not intended to be of direct benefit to the child-subject must be justified in relation to anticipated benefits to society (generalizable knowledge). In general, the risk from*

such interventions should be minimal—that is, no more likely and not greater than the risk attached to routine medical or psychological examination of such children. When an ethical review committee is persuaded that the object of the research is sufficiently important, slight increases above minimal risk may be permitted” [67].

Environmental studies on children using genetic biomarkers have created new ethical issues relating to consent. Consent for the use of data and samples from children must be provided by parents or legal guardians of minors [68,69]. It must also preserve the right of the child to opt in or out of a study, especially when samples are banked for use in new studies several years after collection. This implies that consent must be viewed as an ongoing process and that children recruited into studies at young ages (or prior to their birth) should be able to express their consent routinely as they grow older [70].

Incentives for children to participate in environmental health studies need more consideration. Authorities often claim for altruistic participation, stressing the *feel good* factor, and normally no payment is involved. Some studies choose to donate birthday cards, toys or cinema tickets in return for participation. Teenagers have shown a decreased participation rate in the German Environmental Survey (Bernd Seifert, personal communication) and in several instances ask for compensation claiming study participation is equivalent to work. They compare the hours spent with sampling and interviewing with hours which could have been spent earning money (Ole Wolthers, personal communication).

In December 2003, the CHILDRENGENONET-WORK hosted a meeting on ethical issues related to research with children and the meeting provided the following recommendations: “*Research on and with children is necessary within clinical as well as environmental (public) health in order to provide age-relevant data regarding efficiency and safety of (medicinal) intended treatments and unintended environmental exposures. The stakeholders are: (1) diseased child with interest in optimal treatment (not relevant in public health); (2) children as such with interests of optimal health (UN convention), optimal treatment and no adverse effects from hazardous exposures; (3) parents (family) of children; (4) medical doctors; (5) researchers; (6) society, reg-*

*ulators, administrators, ethical committees, international organisations*”. The program of the meeting, list of participants and several of the presentations is available on the project web site [www.pubhealth.ku.dk/cgn](http://www.pubhealth.ku.dk/cgn).

Research involving children raises specific questions about the study protocol that are to be handled in ethics committees, preferably with input from relevant experts (pediatricians, lawyers, statisticians, toxicologists, psychologists). No best practice in research with children is currently mandated, and the role of ethics committees is mostly regulatory. A direct control over the whole research process is not considered at this time in most countries. However, the best effort should be made to fully explain to children and parents the nature and implications of research.

Informed consent is a prerequisite in all instances—given by parents in case of children of young age (<6 years), and by parents with the concurrence of children in case of school children and adolescents. Important issues surrounding the informed consent include the child’s perception of the information given, perception that may change with age, and the presence of incentives to participate.

The right to withdraw at any time from a research process is fundamental for adults but more equivocal in relation to children. In clinical trials with disease treatment, it may be acceptable to overrule the child’s desire to withdraw based on health benefits; however, in environmental health studies the importance of the child’s will to participate or withdraw should be carefully considered. Further, when the child reaches maturity, his right to withdraw or opt out must be reconsidered and clarified.

Communicating results to parents and/or children must be agreed upon prior to the study. Ethical considerations may be different in cross-sectional compared to longitudinal studies, especially if individual data evaluation and tissue banking have been planned. In environmental studies, direct communication should be given in all studies either at individual or group level, according to the sensitivity of the information (in some cases stigmatisation due to increased disease risk may occur). The child may have the right to be notified of future research plans and whether the decision about future use of data and banked samples will be delegated to the ethics committee.

The ethics committees within Europe and USA are not regulated by the same rules and show wide diversity regarding composition and independence. Further, recommendations by the ethics committees are not standardized and compared even within the same countries.

## 7. Examples from the field

To provide examples of typical problems encountered when studying pediatric populations, we will review three major field studies from Europe and the United States. Study descriptions are brief, but the reader will be introduced to arising issues and referred to the original publications. The first two examples focus on children exposed to specific and relatively well-defined agents. In the third example, a more general assessment of biomarkers of exposure and health effects is conducted in a pediatric population exposed to pesticides and other environmental factors typical for modern agricultural communities.

### 7.1. Chromosomal aberrations (CA) in children exposed to formaldehyde in prefabricated schools

Dobias et al. [71] evaluated the frequency of CA in peripheral blood lymphocytes (PBL) to quantify the amount of genetic damage in children exposed to formaldehyde (FA) in schools. Children were exposed to FA from adhesive used to secure pressboard panels in prefabricated schools in Czechoslovakia in the 1980s. Soon after the schools were built, children started reporting eye and upper respiratory tract

irritations. Chemical analyses revealed high concentrations of FA in classrooms exceeding the maximum allowable concentrations (MAC) of  $35 \mu\text{g}/\text{m}^3$ . Researchers followed children in the elementary (boys, aged 8–12 years) and nursery school (boys and girls, aged 5–6 years). Their findings showed an increased level of CA in children exposed to high FA levels (Table 2). This finding was comparable to cytogenetic damage reported in workers exposed to FA in an occupational setting [72]. These results stimulated the adoption of preventative measures such as wall paneling (wainscoting) to attempt to reduce exposures. Unfortunately, this remedy failed to reduce the frequency of aberrant cells in exposed children in the schools (Table 2). Only gasification of the school with ammonia, which reacts with FA to form the stable compound hexamethylenetetramine, effectively reduced the concentration of FA in air to below the MAC limit. These results clearly demonstrate the clastogenicity of FA in children and show how cytogenetic analysis of PBL in children can be effectively used for biomonitoring and may lead to the development of effective preventative measures, including a ban on FA-releasing panels in kindergartens and schools.

### 7.2. CA in children exposed to air pollution in the Czech Republic

The use of brown coal in metallurgic industry and in power plants in the Czech Republic during the 1980s and early 1990s resulted in serious air pollution that started to decrease only after the mid 90s [73,74]. In the same period, the quality of the typical Czech diet was substantially improved by the increased

Table 2  
Frequency of chromosome aberrations in Czech children with indoor exposure to formaldehyde in different periods of time

Exposure group	Period	Formaldehyde ( $\mu\text{g}/\text{m}^3$ )	Subjects	No. of metaphases	% Aberrant cells (mean $\pm$ S.E.)
School children					
Exposed	1984	317	20	1720	$4.71 \pm 2.09^*$
	1985	130	16	1340	$2.83 \pm 1.64^*$
	1986	36.5	18	1600	$2.06 \pm 1.61$
Referents	1984	–	17	1473	$1.37 \pm 0.89$
	1985	–	17	1653	$2.24 \pm 1.11$
Pre-school children					
Exposed	1984	210–360	13	1300	$2.40 \pm 1.46$
Referents	1984	–	24	2400	$1.12 \pm 1.05$

\*  $P < 0.01$  (exposed 1984 and 1985 vs. referents 1984), modified from Dobias et al. [71].



Table 3  
Frequency of chromosome aberrations detected in Czech children in 1984–1993 and 1994–1999 by age group

Time period	Age (years)	N	% Aberrant cells (mean $\pm$ S.D.)	References
1984–1993	Newborns	129	1.11 $\pm$ 1.14	Bavorová et al. [78]
	4–6	24	1.12 $\pm$ 1.05	Dobiáš et al. [71]
	7–15	196	1.63 $\pm$ 1.18*	Dobiáš et al. [71]; Rössner et al. [75]
	16–19	162	2.02 $\pm$ 1.64*	Rössner et al., [75]; Srb et al. [77]
1994–1999	Newborns	634	1.11 $\pm$ 1.15	Černá et al. [79]; Rössner et al. [75]
	4–6	110	0.59 $\pm$ 0.65	Rössner et al. [75]
	7–15	1885	1.14 $\pm$ 1.15	Černá et al. [79]; Rössner et al. [75]
	16–19	262	1.08 $\pm$ 1.13	Rössner et al. [75]

\*  $P < 0.01$  (period 1984–1993 vs. 1994–1999) modified from [76].

supply of fruits and vegetables, and by supplementation with vitamins and minerals. A study was done to look at the effects of these environmental and dietary improvements on the level of CA in children. Data on lifestyle and measurements of CA were collected from 3402 Czech children aged 0–19 years during the periods 1984–1993 and 1994–1999 [75–79]. A significant decline in chromosomal damage was observed in biological specimens collected from children during the period of 1994–1998 as compared to those collected ten years earlier. Interestingly, the decrease was present in all age groups except newborns (Table 3). These findings demonstrated that CA monitoring in children can be successfully used to evaluate the impact of changes in the levels of air pollution as well as changes in dietary habits.

### 7.3. The CHAMACOS project

The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) is a large project investigating the effects of environmental pollution on children's health in the Salinas Valley in Monterey County, California. The project aims at estimating sources, pathways, levels, and health effects of in utero and postnatal pesticide exposures [80]. Exposure to pesticides is one of the most complex environmental and occupational risks to investigate at a population level, mostly because of the complexity of exposure assessment. The CHAMACOS study population is a marginalized, low-income, mostly Spanish-speaking population of pregnant women and their infants. The combination of these

features creates many challenges that have to be addressed within the study design. Questionnaires and home visits are completed upon enrollment of expectant mothers, at 26 weeks gestation, post delivery, and when the child is 6-, 12- and 24-months-old. Child neurodevelopment and growth assessments are completed during the neonatal period and during home visits. Informed consents signed by both the mother and the father address the issue of future consent to assays in addition to those originally planned, including a clause that stipulates genetic testing. This practice is of particular importance given the mobility of this community, and the difficulty of obtaining biological samples from small children. To date, CHAMACOS has collected over 56,000 biological and environmental specimens, which has created a large biorepository for future research on pregnant women and children [59,80]. Biological and environmental samples have been collected, processed, and stored to maximize the potential for further analysis and to reduce additional research costs. Every biological sample that is collected is separated into several aliquots, each labelled with a unique bar code identifier. Samples of urine, peripheral and cord blood, and breast milk are collected from mothers and their children several times starting with early pregnancy. Urine and home dust are also collected for analysis of exposures to pesticides, allergens, and endotoxin. Cryopreserved blood samples from young children are available for genotoxicity assays. Blood smears can be used for interphase FISH and MN analysis. The study design also includes biomarkers of effect, including immunological measures, cholinesterase activity, and biomarkers of susceptibility, such as paraoxonase

polymorphisms. The coding system does not contain any personal identifying information about the participant as to preserve anonymity of the study subjects. This project has produced extensive documentation on collection, processing, storage, and shipping procedures, including standard operating procedures, chain-of-custody forms, discrepancy reports, and tracking databases. First results of this large study indicate that exposure to organophosphate pesticides is widespread in pregnant women and their newborns and may affect children's growth and development [80].

## 8. Concluding remarks

The critical issues addressed in this paper clearly show that biomarkers of genetic damage may play a major role in understanding and controlling the adverse health effects of environmental pollution in children. The most evident advantage of using biomarkers is the potential for a better exposure assessment in conditions that are difficult to study, such as when exposure occurs at low doses or there is a mixture of toxic substances. Other benefits are more subtle, but their impact on public health may be even more effective. Among these advantages are the possibilities (i) to study gene–environment interaction, (ii) to establish whether a genetic profile can modify individual risk of disease and (iii) to identify subgroups at increased risk. These findings may have the direct consequence of improving the understanding of disease mechanisms. However, the risk of interference with children's emotional equilibrium or a violation of their civil rights resulting from participation in the study should be always considered as the first priority.

In summary, biomonitoring children's genetic damage has a number of risks that must be carefully evaluated in study design. Nevertheless, this approach offers the highest potential for disease prevention and developing regulatory policies.

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