



Review

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

II. Results of a comprehensive literature search and meta-analysis

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Abstract

The present review is based on findings from 178 publications retrieved through an extensive search of the MedLine/PubMed database for a 25 years time period (1980–2004) and 10 manually identified papers. Among the cytogenetic biomarkers that are frequently used in field studies, chromosome aberrations (CA) and micronuclei (MN) but not sister chromatid exchanges (SCE) were found consistently increased in children exposed to environmental pollutants. Meta-analysis of the studies reporting SCE in cord blood showed similar levels of SCE in exposed and in non-exposed newborns. Exposure to airborne pollutants, soil and drinking water contaminants, mostly increased CA and, to a lesser extent, MN levels in children. The effect of exposure to airborne urban pollutants was consistently reported by field studies measuring DNA, albumin and hemoglobin adducts. Prenatal (in utero) and postnatal exposure (environmental tobacco smoke, ETS) to tobacco smoke compounds were associated with

Abbreviations: CA, chromosome aberrations; ELISA, enzyme-linked immunoassay; ETS, environmental tobacco smoke; ETSU, in utero exposure to environmental tobacco smoke; GPA, glycoprotein A; HPRT, hypoxanthine-guanine phosphoribosyltransferase; MeSH, Medical Subject Headings; MN, micronuclei; MR, mean ratio; PAH, polycyclic aromatic hydrocarbons; SAW, soil, air and/or water pollution from industrial or agricultural activities, mines and waste sites, including chemicals indoor and in drinking water; SCE, sister chromatid exchanges; SMKU, in utero exposure to tobacco smoke from smoking mother; UAP, urban air pollution

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increased frequencies of DNA and hemoglobin adducts and CA. The limited number of field studies measuring DNA fragmentation (Comet assay), hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) and the glycophorinA (GPA) mutation frequency in environmentally exposed children precluded a meaningful evaluation of the usefulness of these assays. Meta-analyses performed in children exposed to ETS and in newborns exposed in utero to their mothers' smoke showed 1.3 and 7 times higher levels of hemoglobin adducts compared to referent subjects, respectively. These increases are consistent with the epidemiological evidence of higher lung cancer risks reported in adults who had never smoked and were exposed to ETS during childhood and with 7–15 times higher lung cancer risks reported in smokers than in non-smokers. Higher levels of PAH-DNA adducts were found in fetal than in maternal tissue, suggesting a specific susceptibility of the fetus to this class of ubiquitous environmental pollutants. According to these findings, future research and biomonitoring programs on children would greatly benefit from the inclusion of selected biomarkers that could provide biologically based evidence for the identification of intervention priorities in environmental health.

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1. Introduction

In 2001, the *European Network on children's susceptibility and exposure to environmental genotox-*

icants (CHILDRENGENONETWORK) [1] was planned, which was a research project focusing on the effects of environmental exposure to genotoxic agents during various developmental stages. This

European network collects existing information from participating laboratories and reviews scientific literature to evaluate the evidence of the association between environmental exposure to genotoxic agents during the fetal, neonatal and infancy developmental periods, concentrating on exposures entailed by urban air, soil and water pollution, cigarette smoke and environmental tobacco smoke (ETS), and the level of selected biomarkers of genetic damage. Exposure to ionising radiation (accidental, environmental, therapeutic or diagnostic), a widely studied physical DNA damaging agent, is considered within the project and it will be evaluated separately. The network also explores the use of biomarkers to assess potential cancer risks of environmental exposures. Recommendations concerning the need for new research projects as well as ethical, legal, and social aspects of biomonitoring in children will be delivered to the European Commission and are expected to positively influence European research in the field.

The first step of the present systematic review includes the development of a protocol to conduct meta-analyses [2] on different biomarkers and the establishment of a database for pooled analyses. The following biomarkers were included as intermediate end-points related to the carcinogenesis process: DNA, hemoglobin and albumin adducts (evaluating the biologically effective dose), chromosome aberrations (CA), sister chromatid exchanges (SCE) and micronuclei (MN), DNA fragmentation by the Comet assay (evaluating early biological effect), the hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) and the glycophorinA (GPA) mutation frequency assays (evaluating early biological response).

This review is aimed at identifying field studies conducted among children exposed to chemical environmental pollutants incorporating the above-mentioned biomarkers, evaluating and summarizing the evidence of specific exposure-biomarkers associations. For each study, the ratio of the mean levels (MR = mean ratio) of each biomarker detected in exposed and in referent children was computed as a point estimate of the relative effect of exposure. A more precise quantitative summary estimate of the exposure-biomarker association (meta-MR) was computed when feasible by conducting a meta-analysis to generalize single study findings [3].

2. Materials and methods

2.1. Search strategy

We performed an extensive literature search without any language restriction by using the MedLine/PubMed database (National Library of Medicine, National Institutes of Health, Bethesda, MD, USA-<http://www.ncbi.nlm.nih.gov/PubMed>) and covering the time period between January 1, 1980 and November 30th, 2004. Table 1 shows the keywords selected from Medical Subject Headings (MeSH), (hereafter indicated as keyword [mesh]), and the combinations of search terms that have been used through the Boolean operators. Some of the assays have been developed relatively recently (e.g., Comet assay), and the corresponding MeSH terms have been introduced in the MeSH thesaurus after 1980. In this case, to retrieve articles published before MeSH terms were introduced, we used previously indexed keywords or common research terms along with PubMed *free text* or *phrase search* tools (Table 1).

Individuals from newborns to late adolescence (age 0–18 years) were considered as children. To define the age range of interest the following string search was used: child [mesh] OR infant [mesh] OR adolescent [mesh]. Maternal/fetal exchange [mesh] and fetal blood [mesh] were added to include studies on in utero exposure. Environmental exposure was defined as follows: environmental pollution [mesh] OR environmental pollutants [mesh] OR (smoking [mesh] OR smoke [mesh]) AND (pregnancy [mesh] OR mother [mesh]). The NOT operator was used to exclude most occupational studies and most reports on the effects of ionising radiation (Table 1). Case reports, studies on animals or plants and tutorial reviews were also excluded.

2.2. Inclusion/exclusion criteria

One hundred seventy-eight citations were retrieved through MedLine search, some reporting findings on more than one biomarker, corresponding to 230 citations of a single biomarker. All the articles specifically addressing the association between biomarkers and environmental pollution in children were obtained and manually reviewed. Only papers providing a clear description of the study design and the study findings

Table 1

Keywords [MeSH vocabulary] and search terms (including *phrase search* and *free text* tools) used in MedLine search

Biomarker–assay	Time period ^a	AND operator	NOT operator
Chromosome aberrations [mesh]		Environmental pollution [mesh] Environmental pollutants [mesh]	Occupational diseases [mesh] Occupational medicine [mesh] Occupational exposure [mesh] Air pollutants, occupational [mesh]
Comet assay [mesh] OR Comet [tiab ^c] OR	(2000–2004) (1980–1999)		
Electrophoresis, agar gel [mesh] OR “Single cell gel electrophoresis”	(1980–1999) (1980–1999)	(Smoking [mesh] OR smoke [mesh]) AND (pregnancy [mesh] OR mother [mesh])	Air pollutants, radioactive [mesh] Soil pollutants, radioactive [mesh] Water pollutants, radioactive [mesh] Air pollution, radioactive [mesh] Water pollution, radioactive [mesh]
DNA adducts [mesh] OR “DNA adduct” OR “DNA adducts” OR Adduct [tiab] OR Adducts [tiab]	(1995–2004) (1980–1994) (1980–1994)	Adolescent [mesh] Child [mesh] Infant [mesh] Fetal blood [mesh] Maternal fetal exchange [mesh]	Radioactive waste [mesh] Radioactive pollutants [mesh] Accidents, radiation [mesh]
Hypoxanthine phosphoribosyltransferase [mesh]			Food contamination [mesh]
Glycophorin A [mesh]			Animals [mesh] Plants [mesh]
Sister chromatid exchange [mesh] OR “Sister chromatid exchange” OR “Sister chromatid exchanges”	(1985–2004) (1980–1984) (1980–1984)		Case reports [pt] Review [pt]
Micronucleus test [mesh] OR “Micronucleus test” OR “Micronucleus assay” OR Micronuclei [tiab]	(1989–2004) (1980–1988) (1980–1988)		

mesh, Medical Subject Headings; ^c tiab, title and abstract; pt, publication type.^a If not specified = 1980–2004.

were further considered. Papers lacking a clear definition of the exposure or a reference group were excluded, as were studies including less than 10 children in the exposed and referent groups. Studies including children and adults together were included only when findings regarding children were reported separately. Results of *in vitro* challenge assays were excluded.

2.3. Exposure–biomarker associations

In order to simplify the presentation and the interpretation of the reviewed studies, exposure to environmental pollutants was classified according to the following categories: (i) urban air pollution (UAP), (ii) soil, air and/or water pollution from industrial or agricultural activities, mines and waste sites, including chemicals indoor and in drinking water (SAW), (iii) environmental tobacco smoke, (iv) *in utero* exposure to tobacco smoke from smoking mother (SMKU) and

(v) *in utero* exposure to environmental tobacco smoke (ETSU).

The relationship between environmental exposure(s) and biomarkers of DNA damage was investigated by computing exposure specific ratios (MRs) of the mean levels of each biomarker detected in exposed and in referent children. The MR is a point estimate of the relative effect of the exposure on biomarker level detected in each study taking the value 1 ($MR = 1$) when there is no effect, values greater than 1 ($MR > 1$) when exposure is associated with an increased biomarker level, and lower than 1 ($MR < 1$) when exposure is associated with a decreased level of the investigated biomarker. The MR, as a measure of effect, has the advantage of being independent from the absolute value of the biomarker level reported by the single studies, is comparable across the studies considered, significantly reducing the between-study variability.

2.4. Meta-analysis

When at least three studies investigating the effect of exposure to specific environmental pollutants on a specific biomarker of DNA damage were available, a quantitative summary measure (meta-estimate) of the exposure-biomarker association was computed. To this aim, the study-specific MRs were log transformed to normalize their distribution and weighted according to the variance of each study-specific MR [4]. A formal meta-analysis was performed using the random effects model [5], which allows for an estimate of a meta-MR and its 95% confidence interval by using Stata statistical software [6].

2.5. Differential susceptibility

The issue of an increased susceptibility of children and fetuses to environmental genotoxic agents [7–9] was addressed by computing exposure-specific maternal/fetal and adults/children ratios (MRs) of the mean levels of each biomarker detected in exposed and in referent subjects, respectively. A maternal/fetal MR ≤ 1 was considered to be clearly supportive of a higher susceptibility of the developing fetus, given the experimental evidence indicating that the transplacental dose of genotoxic compounds (e.g., PAHs) to the fetus is an order of magnitude lower than the dose to maternal tissues [10–12]. A maternal/fetal MR > 1 (reflecting higher levels of DNA damage in mothers than in newborns) was considered inconclusive

considering the maternal/fetal dose and the expected (and unknown) DNA damage differentials, as well as the differing abilities to metabolise genotoxic agents, DNA repair capacity, and rates of cell proliferation [7,9–12]. A lower maternal/fetal ratio in exposed than in referent subjects (measuring, for the latter, the ratio of biomarker baseline levels in maternal and in fetal tissues), was considered as supportive of a higher susceptibility of children to genotoxic compounds.

The same criteria were followed to evaluate the adults/children MRs, although postnatal exposure to environmental genotoxic compounds and the adults/children comparison are likely to be more complex than for mothers–newborns pairs and the interpretation of these MRs less straightforward.

3. Results

3.1. Exposure-biomarker associations

The MedLine search strategy retrieved 178 studies. Cytogenetic biomarkers (CA, SCE and MN), were the most frequently found biomarkers through MedLine search (Table 2). CA were mentioned in 78 studies, SCE in 38 and MN in 27. Thirty-two studies measuring DNA adducts, 14 and 10 measuring hemoglobin and albumin adducts, respectively, were retrieved. The Comet assay was cited in 18 studies, the *HPRT* loci mutant frequency assay in 11, and the GPA human mutation assay in two field studies. The

Table 2

Studies evaluating the association between biomarkers of genetic damage in children exposed to environmental pollutants retrieved through MedLine search, manual examination, and studies included in the review

Biomarker–assay	MedLine search		Manual examination	Total included
	Retrieved No.	Included No. (%)	Included No.	No.
Chromosome aberrations	78	2 (2.6)	4	6
Sister chromatid exchanges	38	14 (36.8)	1	15
Micronuclei	27	4 (14.8)	2	6
DNA adducts	32	5 (15.6)	2	7
Haemoglobin adducts	14	7 (50.0)	2	9
Albumin adducts	10	4 (40.0)	–	4
Comet assay	18	7 (38.9)	1	8
<i>HPRT</i> mutation frequency	11	5 (45.5)	–	5
GPA mutation frequency	2	1 (50.0)	–	1
Total ^a	178	39 (22.0)	10	49

^a The sum does not add up to total since studies can report findings for more than one biomarker.

efficacy of the MedLine search was particularly low for the chromosome aberrations [mesh] term, with 43 out of 78 studies retrieved (55%) unsuitable for inclusion (23 reporting chromosomal abnormalities in spontaneous abortions and congenital disorders, 11 assessing MN and SCE, and 9 investigating exposure to ionising radiation). They were all excluded after manual inspection, leaving 35 CA papers for a possible inclusion in the review (not shown in Table 1).

Of the 178 MedLine retrieved studies, 39 met the inclusion criteria and were included in the review. Ten additional studies were manually identified (Table 2) through the examination of the references reported by the MedLine-retrieved studies included in the systematic review, resulting in a total number of 49 studies [13–61].

Peripheral or cord blood were the most commonly used biological specimens, with only 3 studies evaluating biomarkers in cells from oral or nasal mucosa [20,23,36].

The studies characteristics and their findings are shown by type of assay and, within each assay, by category of exposure to environmental pollutants in Tables 3–10. For each study we reported main design features (type and source of exposure, size of the exposed and referent groups, range or mean age of study subjects), main findings (mean level \pm standard error (S.E.) of biomarker detected in exposed and referent children, the statistical comparison as reported in the original publication, the computed exposed-referent MRs, the first author and the year of publication. When relevant data on exposure and other individual characteristics were reported separately and were used for the interpretation of the observed findings, first author and the year of publication are indicated in parenthesis. For those studies measuring biomarkers in more than one group of exposed children (i.e., reporting data for subgroups exposed to different levels of the same environmental pollutants), biomarker(s) mean values and the corresponding MR are always reported for the subgroup with the highest level of exposure [17,19,22,23,37,38,45,53,61]. In addition, subgroups were collapsed assuming their independency and the biomarker mean level and the corresponding standard error, weighted for the size of each subgroup, were estimated and reported in the tables. Accordingly, MRs for collapsed data were computed and reported.

3.2. Chromosome aberrations

Most studies including CA as a biomarker [13–18] detected increased frequencies in exposed children (Table 3). Specifically, environmental pollution from industrial activities, chemical waste sites, formaldehyde and toluene indoor pollution, and NO₃ polluted drinking water were significantly associated with an increased frequency of CA in 4 (67%) out of 6 studies. Up to five-fold increased MR was detected in Lithuanian children (MR = 5.32) living downwind of chemical plants [14] and in Greek children (MR = 4.83) exposed to NO₃ contaminated drinking water [18] compared to referent subjects. CA frequency was slightly decreased (MR = 0.89) in one study investigating the effect of urban air pollution from residential heating in the Czech Republic [13].

3.3. Micronuclei

All the studies conducted in children exposed to environmental pollutants revealed clearly increased MN frequencies in exposed compared to referent children (Table 4). Children exposed to urban air pollutants in Moscow [19] and St. Petersburg [20] had twice higher levels of MN than referents (MR = 2.28 and 2.13, respectively). Children exposed to airborne pollutants in Calcutta had a 30% increased level of MN (MR = 1.29) compared to residents in rural areas [21]. MN frequency was increased in children living downwind of a chemical disposal site (MR = 2.38) [22], in infants exposed to heavy metals and other industrial pollutants (MR = 7.25) [23], and in subjects exposed to high levels of arsenic in drinking water (MR = 6.25) [24]. A 30% increased MN frequencies (MR = 1.29) was accounted by indoor exposure to ETS from smoking parents [25]. All the increased MN levels were statistically significant.

3.4. Sister chromatid exchanges

SCE were evaluated as a biomarker of exposure to environmental pollutants in 11 studies [14,18,22,24,26–35] (Table 5). Two studies included children exposed to soil, air and water pollutants due to the presence of chemical industry and disposal site [14,22], two children exposed to chemicals in drinking water [18,24] and one residents of lead

Table 3

Frequency of chromosome aberrations detected in peripheral blood or cord blood from children (0–18 years old) by type of exposure to environmental pollutants and ratio (MR) of the mean level detected in exposed relative to referent children

Exposure type ^a (Country)	Exposure definition/source Referents definition	Groups Sample size	Age (years) ^b	Mean \pm S.E. ^c	MR	First author, year
UAP (Czech Republic)	Industrial + residential heating (Teplice)	Exposed = 86	Newborns	0.89	0.89	Šrám, 1999
	Residents in agricultural districts (Prachatice)	Referents = 29		1.00	1	
SAW (Lithuania)	Residents 10 km downwind of chemical plants Rural inhabitants	Exposed = 15	11–15	2.18 \pm 0.3*	5.32	Lazutka, 1999
		Referents = 21		0.41 \pm 0.11	1	
SAW (Czech Republic)	Indoor formaldehyde (school facilities) Scholars attending unpolluted facilities	Exposed = 20	8–12	4.71 \pm 2.09*	3.44	Dobiáš, 1988
		Referents = 17		1.37 \pm 0.89	1	
SAW (Czech Republic)	Indoor formaldehyde + toluene (school facilities) Scholars attending unpolluted facilities	Exposed = 22	15–18	3.67*	1.66	Srb, 1990
		Referents = 21		2.21	1	
SAW (Belgium)	Indoor formaldehyde only (school facilities) Scholars attending unpolluted facilities	Exposed = 21	15–18	3.07*	1.20	
		Referents = 21		2.55	1	
SAW (Belgium)	Residents near Pb smelters, waste incinerators Residents in rural area	Exposed = 38	17.2 \pm 0.8	76.21 \pm 6.98 ^{d,c}	1.06	Staessen, 2001
		Referents = 36		72.0 \pm 7.48	1	
SAW (Greece)	Drinking water highly polluted with NO ₃ Residents in non-agricultural area	Exposed = 19	12–15	2.9 \pm 3.1*	4.83	Tsezou, 1996
		Referents = 20		0.6 \pm 0.9	1	

^a UAP, urban air pollution; SAW, soil, air and water pollution, ETS, environmental tobacco smoke; ETSU, ETS in utero; SMKU, smoking in utero.

^b Range values or mean \pm S.E.

^c Mean value \pm S.E. (when reported by the original paper).

^d Mean value \pm S.E. computed from collapsed subgroups, assuming their independency, and weighted for the variance of each subgroup.

^e Defined as % children with ≥ 1 lymphocytes with CA.

* Statistically significant difference between means, as indicated in the original paper.

Table 4

Frequency of micronuclei detected in peripheral blood or buccal cells^a from children (0–18 years old) by type of exposure to environmental pollutants and ratio (MR) of the mean level detected in exposed relative to referent children

Exposure type ^b (Country)	Exposure definition/source Referents definition	Groups Sample size	Age (years) ^c	Mean \pm S.E. ^d	MR	First author, year
UAP (USSR)	Air pollutants in three Moskow districts Residents in unpolluted areas	Exposed = 145 Referent = 473	2–16	17.8 \pm 1.3 ^{*,c} 7.8 \pm 0.2	2.28 1	Pelevina, 2001
UAP (USSR)	Highly polluted S. Petersburg district Three polluted S. Petersburg districts Less polluted S. Petersburg district	Exposed = 31 Exposed = 73 Referent = 38	1–6	8.1 [*] 6.6 ^{nr} 3.8	2.13 1.74 1	Maimulov, 1998
UAP (India)	Air pollutants (traffic and factories) in Calcutta Residents in rural areas	Exposed = 153 Referent = 116	6–17	2.2 \pm 0.4 [*] 1.7 \pm 0.3	1.29 1	Lahiri, 2000
SAW (Belgium)	Residents downwind of chemical disposal site Residents near chemical waste site Residents in semi rural area	Exposed = 12 Exposed = 45 Referent = 33	6–16	7.00 \pm 1.12 [*] 4.78 \pm 0.97 2.94 \pm 0.43	2.38 1.63 1	Vleminckx, 1997
SAW (USSR)	Heavy metals and chemical industrial pollution Resident in rural areas	Exposed = 221 Referent = 56	1–6	8.7 \pm 0.2 ^{*,c} 1.2 \pm 0.1	7.25 1	Gorovaia, 2002
SAW (Argentina)	High levels of arsenic in drinking water Low levels of arsenic in drinking water	Exposed = 10 Referent = 12	8 8	35.0 \pm 4.6 [*] 5.6 \pm 1.6	6.25 1	Dulout, 1996
ETS (Germany)	ETS from smoking parents ETS unexposed	Exposed = 45 Referent = 32	3–15	8.0 [*] 6.2	1.29 1	Baier, 2002

The superscript "nr" represents statistical comparison not reported.

^a Buccal epithelium cells: Maimulov, 1998, Gorovaya, 2002.

^b See footnote Table 3.

^c Range values or mean \pm S.E.

^d Mean value \pm S.E. (when reported by the original paper).

^e Mean value \pm S.E. computed from collapsed subgroups, assuming their independency, and weighted for the variance of each subgroup.

* Statistically significant difference, as indicated in the original paper.

contaminated areas [28]. One study included children with smoking parents exposed indoor to ETS [29], and five focused on the effect of in utero exposure to cigarette smoke by studying newborns whose mothers smoked during pregnancy [30–35]. Similar SCE frequencies in exposed and referent children were reported by most of the studies, with MRs close to 1. Two studies in children exposed to NO₃ [18] and arsenic [24] in drinking water reported slightly reduced SCE mean levels than in referents (MR = 0.89–0.92 and 0.96, respectively). Conversely, a two-fold higher SCE level was detected in children living downwind of a chemical disposal site (MR = 2.12) [22]. Only one out of five studies conducted in newborns exposed in utero to cigarette smoke found a statistically significant increased level of SCE compared to non-exposed newborns (MR = 1.36) [33], all the others reporting MRs close to 1.

3.5. DNA adducts

DNA adducts were measured in children exposed to environmental pollution in three populations (Table 6) [17,36–38]. A significant increase was detected in nasal biopsy of children exposed to urban air pollutants in the Metropolitan Mexico City (MR = 2.99) [36] and in cord blood from newborns (MR = 4.79) of unemployed mothers who lived in polluted areas of the city of Crakow, Poland [37,38]. Higher levels of DNA adducts were reported in adolescents living in a suburb highly polluted with organic volatile compounds in Belgium (MR = 1.30) compared to referents living in a rural area [17]. In utero exposure to cigarette smoke compounds was investigated in three populations. Smoking 5–30 cigarettes per day during pregnancy was associated with a significantly increased DNA adducts level in cord blood of newborns (MR = 10.66) [40], while

Table 5
Frequency of sister chromatid exchanges in peripheral or cord blood cells from children (0–18 years old) by type of exposure to environmental pollutants and ratio (MR) of the mean level detected in exposed relative to referent children

Exposure type ^a (Country)	Exposure definition/source Referents definition	Groups Sample size	Age (years) ^b	Mean \pm S.E. ^c	MR	First author, year
SAW (Lithuania)	Residents 10 km downwind of chemical plants Rural inhabitants	Exposed = 15	11–15	7.76 \pm 0.21	1.06	Lazutka, 1999
		Referents = 21		7.29 \pm 0.21	1	
SAW (Belgium)	Residents downwind of chemical disposal site Residents in semi rural area	Exposed = 22	3–16	12.44 \pm 1.52 ^{d,nr}	2.12	Vleminckx, 1997 (Laurent,1993); (Lakhanisky,1993)
		Referents = 31		5.87 \pm 0.15	1	
SAW (Italy)	Residents near a smelter (lead pollution) Residents in a non-contaminated area	Exposed = 19	3	9.0	1.01	Dalprà, 1983
		Referents = 12	6	8.9	1	
SAW (Greece)	High levels of NO ₃ in drinking water Intermediate levels of NO ₃ in drinking water Low levels of NO ₃ in drinking water Residents in non-agricultural area	Exposed = 19	12–15	7.77 \pm 0.14	0.92	Tsezou, 1996
		Exposed = 18		7.36 \pm 0.17 [*]	0.89	
		Exposed = 33		7.46 \pm 0.16 [*]	0.89	
		Referents = 20		8.41 \pm 0.27	1	
SAW (Argentina)	High levels of arsenic in drinking water Low levels of arsenic in drinking water	Exposed = 8	8	4.4 \pm 1.1	0.96	Dulout, 1996
		Referents = 16	8	4.6 \pm 1.2	1	
ETS (USA)	Smoking mothers Non-smoking mothers	Exposed = 32	1–6	10.07 \pm 2.28	1.14	Tang, 1999
		Referents = 11		8.82 \pm 1.78	1	
SMKU (USA)	Mothers smoking 8–40 cigarettes per day Non-smoking mothers	Exposed = 21	Newborns	9.7 \pm 0.38	1.01	Lundgren, 1987
		Referents = 22		9.6 \pm 0.56	1	
SMKU (Finland)	Mothers smoking Non-smoking mothers	Exposed = 17	Newborns	6.1 \pm 0.5	1.03	Sorsa, 1988 (1989)
		Referents = 25		5.9 \pm 0.5	1	
SMKU (Turkey)	Mothers smoking \geq 1 cigarettes per day Non-smoking mothers	Exposed = 21	Newborns	5.20 \pm 0.24 [*]	1.36	Şardaş, 1995
		Referents = 10		3.83 \pm 0.14	1	
SMKU (Australia)	Mothers smoking \geq 5 cigarettes per day Non-smoking mothers	Exposed = 25	Newborns	9.01 \pm 0.28	1.01	Seshadri, 1982
		Referents = 30		8.95 \pm 0.37	1	
SMKU (Italy)	Mothers smoking \geq 5 cigarettes per day Non-smoking mothers	Exposed = 10	Newborns	5.74 \pm 0.76	0.97	Ardito, 1980
		Referents = 10		5.94 \pm 0.73	1	

The superscript “nr” represents statistical comparison not reported.

^a See footnote Table 3.

^b Range values or mean \pm S.E.

^c Mean value \pm S.E. (when reported by the original paper).

^d Mean value \pm S.E. computed from collapsed subgroups, assuming their independency, and weighted for the variance of each subgroup.

* Statistically significant difference, as indicated in the original paper.

Table 6

Frequency of DNA adducts in peripheral or cord blood cells and in nasal biopsies^a from children (0–18 years old) by type of exposure to environmental pollutants and ratio (MR) of the mean level detected in exposed relative to referent children

Exposure type ^b (Country)	Exposure definition/source Referents definition	Groups Sample size	Age (years) ^c	Mean \pm S.E. ^d	MR	First author, year
UAP (Mexico)	Airborne pollutants (Metropolitan Mexico City)	Exposed = 86	6–13	627 ^{e,*}	2.99	Calderón-Garcidueñas, 1999
	Low polluted coastal town (Manzanillo)	Referents = 12	10.8	210 \pm 35.2	1	
UAP (Poland)	High + Medium polluted areas (Crakow)	Exposed = 40	Newborns	5.95 \pm 1.15 ^{f,nr}	1.06	Whyatt, 1998 (Perera,1999)
	Low polluted areas (Crakow)	Referents = 17		5.60 \pm 2.1	1	
	High + Medium (Crakow-unemployed mothers)	Exposed = 17	Newborns	8.14 \pm 1.96 ^{f,*}	4.79	
	Low polluted areas (Crakow-unemployed mothers)	Referents = 6		1.7 \pm 0.5	1	
SAW (Belgium)	Benzene/toluene highly polluted suburb	Exposed = 42	17.2 \pm 0.1	0.57 \pm 0.02 [*]	1.30	Staessen, 2001
	Cd smelters, waste incinerators (2 suburbs)	Exposed = 100	17.5 \pm 0.1 ^g	0.52 \pm 0.028 ^{f,nr}	1.18	
	Rural area	Referents = 100	17.2 \pm 0.1	0.44 \pm 0.02	1	
ETSU (USA)	Non-smoking mothers exposed to ETS	Exposed = 100	Newborns	0.24 \pm 0.02	1.00	Perera, 2004
	Non-smoking mothers not exposed to ETS	Referents = 165		0.24 \pm 0.02	1	
ETSU (Poland)	Non-smoking mothers exposed to ETS	Exposed = 17	Newborns	6.1 \pm 1.7 ^{nr}	1.24	Whyatt, 1998 (Perera,1999)
	Non-smoking mothers not exposed to ETS	Referents = 15		4.9 \pm 1.8	1	
SMKU (France)	Mothers smoking 5–30 cigarettes per day	Exposed = 15	Newborns	11.62 \pm 1.71 [*]	10.66	Arnould, 1997
	Non-smoking mothers	Referents = 10		1.09 \pm 0.99		
SMKU (Poland)	Mothers smoking up to delivery	Exposed = 7	Newborns	6.1 \pm 3.7 ^{nr}	1.09	Whyatt, 1998 (Perera,1999)
	Mothers quitting before delivery	Exposed = 19		5.9 \pm 2.1 ^{nr}	1.05	
	Non-smoking mothers	Referents = 32		5.6 \pm 1.2	1	
SMKU (Poland)	Mothers smoking	Exposed = 11	Newborns	14.7 \pm 4.13 ^{nr}	0.86	Whyatt, 2001
	Non-smoking mothers	Referents = 79		17.1 \pm 1.27	1	

The superscript “nr” represents statistical comparison not reported.

^a Calderon-Garcidueñas, 1999.

^b See footnote Table 3.

^c Range values or mean values \pm S.E.

^d Mean value \pm S.E. (when reported by the original paper).

^e Median of subgroups’ mean values.

^f Mean value \pm S.E. computed from collapsed subgroups, assuming their independency, and weighted for the variance of each subgroup.

^g Mean \pm S.E. computed from collapsed subgroups.

^{*} Statistically significant difference, as indicated in the original paper.

Table 7

Frequency of haemoglobin adducts in peripheral or cord blood cells from children (0–18 years old) by type of exposure to environmental pollutants and ratio (MR) of the mean level detected in exposed relative to referent children

Exposure type ^a (Country)	Exposure definition/source Referents definition	Groups Sample size	Age (years) ^b	Mean \pm S.E. ^c	MR	First author, year
UAP (Italy)	City suburbs residents	Exposed = 61	3–15	5.70 \pm 2.05	1.33	Bono, 2005 ^e
	Residents in rural villages	Referents = 39		4.29 \pm 1.96	1	
ETS (Italy)	ETS exposed, living in urban settings	Exposed = 33	3–13	10.78 \pm 5.34 [*]	4.08	Bono, 2005
	ETS unexposed, living in urban settings	Referents = 24		2.64 \pm 1.05	1	
	ETS exposed, living in rural settings	Exposed = 18	3–13	6.79 \pm 2.67 ^{nr}	3.07	
	ETS unexposed living in rural settings	Referents = 21		2.21 \pm 0.48	1	
ETS (Germany)	ETS from smoking parents	Exposed = 34	3–15	82.2 [*]	1.36	Baier, 2002
	ETS unexposed	Referents = 29		60.6	1	
ETS (Germany)	Mother or other smokers in household ETS unexposed	Exposed = 58 Referents = 65	6–7	28.5 \pm 2.63 25.0 \pm 1.83	1.14	Richter, 2001
ETS (USA)	Mothers smoking 1–30 cigarettes per day	Exposed = 23	1–6	35.9 \pm 4.03 [*]	1.51	Tang, 1999
	Non-smoking mothers; other smokers in house	Exposed = 18		32.4 \pm 3.16 [*]	1.36	
	ETS unexposed	Referents = 10		23.8 \pm 2.92	1	
SMKU (Portugal)	Mothers smoking 15–30 cigarettes per day	Exposed = 13	Newborns	147 \pm 18.86 [*]	3.50	Tavares, 1994
	Non-smoking mothers	Referents = 10		42 \pm 5.69	1	
SMKU (USA)	Mothers smoking >40 cigarettes per day	Exposed = 20	Newborns	319 \pm 11.0 [*]	35.92	Myers, 1996 (Pinorini-Godly, 1996)
	All smoking mothers	Exposed = 74		184 \pm 11.6 [*]	20.72	
	Mothers smoking <40 cigarettes per day	Exposed = 54		134 \pm 11.7 ^{nr,d}	15.09	
	Non-smoking mothers	Referents = 74		8.88 \pm 0.67	1	
SMKU (USA)	Mothers smoking	Exposed = 14	Newborns	92 \pm 14.43 [*]	5.41	Coghlin, 1991
	Non-smoking mothers	Referents = 38		17 \pm 2.11	1	
SMKU (Denmark)	Mothers smoking	Exposed = 20	Newborns	55.2 [*]	7.56	Farmer, 1996
	Non-smoking mothers	Referents = 29		7.3	1	

The superscript “nr” represents statistical comparison not reported.

^a See footnote Table 3.

^b Range values or mean \pm S.E.

^c Mean value \pm S.E. (when reported in the original paper).

^d Mean value \pm S.E. computed from collapsed subgroups, assuming their independency, and weighted for the variance of each subgroup.

^e Available as advance online publication, 17 march 2004; doi:10.1038/sj.jea.7500344, in 2004.

* Statistically significant difference between means, as indicated in the original paper.

Table 8
Frequency of albumin adducts in peripheral or cord blood cells from children (0–18 years old) by type of exposure to environmental pollutants and ratio (MR) of the mean level detected in exposed relative to referent children

Exposure type ^a (Country)	Exposure definition/source Referents definition	Sample size	Age (years) ^b	Mean \pm S.E. ^c	MR	First author, year
UAP (Denmark)	Residents in Aarhus urban area Residents in Aarhus suburban area Residents in Aarhus rural areas	Exposed = 40 Exposed = 37 Referents = 30	Newborns	Higher mean levels in newborns delivered by urban non-smoking mothers ^{d,nr}	nc nc	Autrup, 1995 (1996)
SMKU (Denmark)	Mothers smoking Non-smoking mothers	Exposed = 21 Referents = 107	Newborns	Higher mean levels in newborns delivered by smoking mothers ^{d,nr}	nc	Autrup, 1995 (1996)
ETS (USA)	Mothers smoking 1–30 cigarettes per day Non-smoking mothers, other smokers in house ETS unexposed	Exposed = 44 Exposed = 38 Referents = 24	1–6	$0.54 \pm 0.09^*$ 0.32 ± 0.07 0.19 ± 0.03	2.84 1.68 1	Tang, 1999 (Crawford, 1994)

nc: MR not computable. The superscript “nr” represents statistical comparison not reported.

^a See footnote Table 3.

^b Range values or mean \pm S.E.

^c Mean value \pm S.E. (when reported in the original paper).

^d Graphically depicted data only.

* Statistically significant difference between means, as indicated in the original paper.

smoking >1 cigarette per day throughout pregnancy or quitting 1 month or more prior to delivery resulted in slightly increased DNA adduct levels in newborns (MR = 1.09 and 1.05, respectively) [37,38]. One study reported a slight reduction of DNA adducts in newborns whose mother smoked up to delivery (MR = 0.86) [41]. Exposure to ETS in utero resulted in higher DNA adducts levels in Polish [37,38] but not in African–American newborns [39], with MR = 1.24 and 1, respectively.

3.6. Hemoglobin adducts

Hemoglobin adducts (Table 7) were measured in nine studies conducted in children exposed to urban air pollutants [42], ETS [25,29,42,43], and in newborns exposed to smoking in utero [44–48]. Exposure to urban airborne pollutants experienced by city suburb residents was associated with a 30% increased level of hemoglobin adducts (MR = 1.33) when compared to residents in rural villages [42]. Increased mean levels of hemoglobin adducts were consistently reported in children exposed to ETS, with MRs ranging between 1.14 [43] and 4.08 [42]. Fetal exposure to maternal smoking was consistently associated with increased hemoglobin adducts levels, with MRs ranging between 3.50 and 35.92 for mothers who smoked 15–30 and >40 cigarettes per day during pregnancy, respectively [44,45].

3.7. Albumin adducts

Albumin adducts (Table 8) were measured in newborns exposed in utero to smoking and urban air pollutants [49,50] and in infants exposed to ETS [29,51]. Higher levels were reported in newborns delivered by smoking mothers compared to non-smoking mothers and in newborns delivered by non-smoking mothers residents in Aarhus (Denmark) urban area compared to residents in rural areas [49,50]. Children exposure to ETS was associated with a significantly increased level of albumin adducts in children whose mothers smoked 1–30 cigarettes per day (MR = 2.84), and in children of non-smoking mothers exposed to ETS from in household smokers (MR = 1.68) [29,51].

Table 9

DNA damage assessed by the Comet assay in peripheral or cord blood cells from children (0–18 years old) by type of exposure to environmental pollutants and ratio (MR) of the mean level detected in exposed relative to referent children

Exposure type ^a (Country)	Exposure definition/source Referents definition	Groups Sample size	Age (years) ^b	Mean \pm S.E. ^c	MR	First author, year
UAP (Czech Republic)	Air pollution from residential heating (Teplice)	Exposed = 211	Newborns	5.26 \pm 0.09	0.94	Šrám, 1998 (1999)
	Residents in agricultural districts (Prachatic)	Referent = 137		5.59 \pm 0.12	1	
UAP (Mexico)	Airborne pollutants (Metropolitan Mexico City)	Exposed = 129	6–12	82.16 \pm 6.4	4.83	Calderón–Garcidueñas, 1997 (1996, 1999)
	Low polluted coastal town (Manzanillo)	Referent = 19	11	17 \pm 6.07	1	
SAW (Mexico)	Living close to a mining site (arsenic and lead)	Exposed = 20	3–6	67.6 \pm 5.59 ^{d,*}	1.62	Yáñez, 2003
	Living 15 km away, upwind of the mining site	Referent = 35		41.7 \pm 3.40	1	
SAW (Belgium)	Living close to a mining site (arsenic and lead)	Exposed = 20	17.2 \pm 0.1	6.8 \pm 1.0 ^{e,*}	2.13	Staessen, 2001
	Living 15 km away, upwind of the mining site	Referent = 35		3.2 \pm 0.34	1	
SAW (Belgium)	Benzene/toluene highly polluted suburb	Exposed = 42	17.2 \pm 0.1	1.70 \pm 0.08 [*]	1.67	Staessen, 2001
	Cd smelters and waste incinerators (2 suburbs)	Exposed = 100	17.5 \pm 0.1 ^f	1.30 \pm 0.05 ^{g,nr}	1.27	
	Rural area	Referent = 100	17.2 \pm 0.1	1.02 \pm 0.04	1	
SAW (Belgium)	Residents in semi rural area near a waste site	Exposed = 12	4	0.30 \pm 0.02	0.91	Klemans, 1995
	Residents in semi rural village	Referents = 34	6	0.33 \pm 0.03	1	

The superscript “nr” represents statistical comparison not reported.

^a See footnote Table 3.

^b In years, range values or mean \pm S.E.

^c Mean value \pm S.E. (when reported by the original paper).

^d Tail length.

^e Tail moment.

^f Mean \pm S.E. computed from collapsed data.

^g Mean value \pm S.E. computed from collapsed subgroups, assuming their independency, and weighted for the variance of each subgroup.

* Statistically significant difference between means, as indicated in the original paper.

Table 10

Frequency of mutant *HPRT* and *GPA* in cord blood cells from newborns by type of exposure to environmental pollutants and ratio (MR) of the mean level detected in exposed relative to referent children

Exposure type ^a (Country)	Exposure definition/source Referents definition	Groups Sample size	Age (years) ^b	Mean \pm S.E. ^c	MR	First author, year
SMKU (USA, <i>HPRT</i>)	Mothers smoking ≥ 4 cigarettes per day Non-smoking mothers	Exposed = 10 Referents = 9	Newborns	2.17 \pm 0.24* 0.77 \pm 0.13	2.82 1	Ammenheuser, 1994
SMKU (USA, <i>HPRT</i>)	Mothers smoking Non-smoking mother exposed to ETS Non-smoking mothers not exposed to ETS	Exposed = 12 Exposed = 21 Referents = 21	Newborns	0.71 \pm 0.15 1.13 \pm 0.31 0.71 \pm 0.11	1.00 1.59 1	Finette, 1997 (1998)
SMKU (USA, <i>HPRT</i>)	Mothers smoking Non-smoking mothers	Exposed = 13 Referents = 17	Newborns	0.78 \pm 0.13 ^d 0.86 \pm 0.19	0.92 1	Bigbee, 1999
SMKU ETSU (USA, <i>HPRT</i>)	Mothers smoking Non-smoking mothers (20/28 exposed to ETS) Referents not exposed to ETS	Exposed = 27 Exposed = 28 Referents = 8	Newborns	1.4 \pm 0.19* 1.4 \pm 0.25 0.8 \pm 0.18	1.75 1.75 1	Manchester, 1995
SMKU (USA, <i>GPA</i>)	Mothers smoking Non-smoking mothers not exposed to ETS	Exposed = 58 Referents = 18	Newborns	4.1 \pm 0.28 3.6 \pm 0.61	1.14 1	Manchester, 1995

^a See footnote Table 3.

^b Range values or mean \pm S.E.

^c Mean value \pm S.E. (when reported by original paper).

^d Mean value, assay = 0 values excluded.

* Statistically significant difference between means, as indicated in the original paper.

3.8. Comet assay

DNA damage assessed by Comet assay (Table 9) was significantly increased in children exposed to urban airborne pollutants in Metropolitan Mexico City (MR = 4.83) [36,53,54], in children living close to a mining site and exposed to arsenic and lead in soil, air and water (MR = 1.62 for tail length, MR = 2.13 for tail moment) [55], and in adolescents living in suburb polluted by volatile compounds (MR = 1.67) [17]. Exposure to air pollutants from residential heating in Teplice (Czech Republic) was not found to induce any increase in DNA damage compared to children who lived in the less polluted agricultural district of Prachatice (MR = 0.94) [13,52]. The mean level of DNA damage in children exposed to air pollutants from a waste site [56] was similar to that reported for children of semi rural villages (MR = 0.91).

3.9. *HPRT* and *GPA* assays

Increased levels of the *HPRT* loci mutant frequencies were detected in three out of four studies included in the review (Table 10). The *HPRT* mutant frequency was significantly increased in newborns

whose mothers actively smoked during pregnancy (MR = 1.75) with a high level detected in smokers of ≥ 4 cigarettes per day (MR = 2.82) compared to referent newborns [57,60]. One study detected slightly decreased *HPRT* mutant frequency in newborns delivered by mothers smoking throughout pregnancy (MR = 0.92) compared to those who have never smoked [61], and one failed to report any increase of *HPRT* mutant frequencies in newborns exposed to cigarette smoke in utero (MR = 1.0) [58]. Increased *HPRT* mutant frequency (MR = 1.59, 1.75) were detected in newborns of non-smoking mothers exposed to ETS [59,60]. A slight increase was found in the single study measuring *GPA* mutant frequency in newborns whose mothers smoked during pregnancy (MR = 1.14) compared to referent newborns [60].

3.10. Meta-analyses

Meta-analysis was conducted for the two most commonly investigated homogeneous environmental exposures among the studies considered: in utero exposure to smoking and postnatal exposure to ETS. For these exposures, a sufficient number of studies was available only for hemoglobin adducts [25,29,

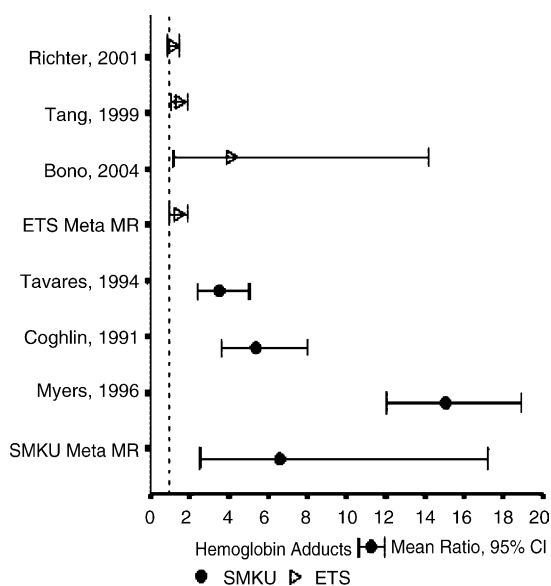


Fig. 1. Hemoglobin adducts meta-mean ratio (SMKU meta-MR) computed in newborns exposed to cigarette smoke in utero and in children exposed to environmental tobacco smoke (ETS meta-MR).

42–45,47,48] and SCE [30,31,33–35]. Two studies measuring hemoglobin adducts were excluded from the meta-analysis because the variance of the mean levels in exposed and referents subjects was not reported in the original publications [25,48]. The results of the meta-analyses are shown in Figs. 1 and 2. The hemoglobin adducts meta-mean ratio (meta-MR) estimates were 1.38 (95%CI = 0.98–1.96) for postnatal exposure to ETS, and 6.65 (95%CI = 2.56–17.24) for in utero exposure to smoking (Fig. 1). The estimated meta-MR for SCE was 1.02 (95%CI = 0.94–1.10), well in line with the MRs reported by the single studies (Fig. 2).

3.11. Comparison of biomarkers level in children and adults

Biomarkers of genetic damage in children and adults exposed to the same environmental pollutants were available in four populations (Table 11) [24,26,27,51,53]. SCE frequency was investigated in children and adults exposed to soil, air and water pollutants who lived downwind of a chemical disposal site in Belgium [26,27], MN frequency in Argentinians exposed to high levels of arsenic in drinking water [24], albumin adducts in ETS exposed Hispanic and African-American children and non-smoking mothers [51],

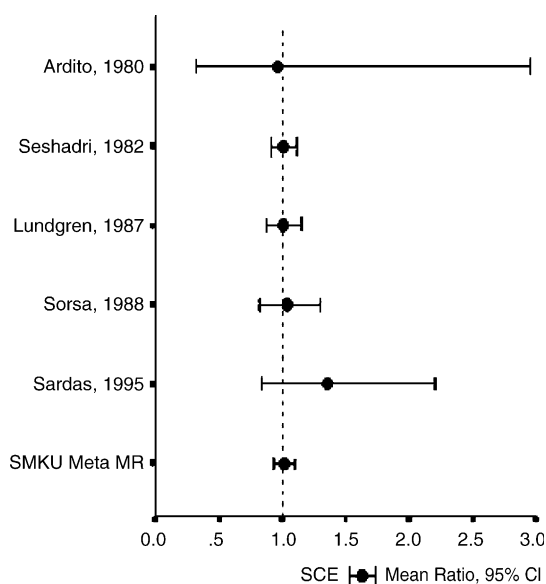


Fig. 2. Sister chromatid exchange meta mean ratio (SMKU meta-MR) computed in newborns exposed to cigarette smoke in utero.

and DNA damage (Comet assay) in Mexico City residents exposed to urban air pollutants [53]. The ratios of the biomarker mean levels detected in adults to those in children (MRs), in exposed and referent subjects are shown in Table 11. The extent of DNA damage assessed by the Comet assay and the DNA-surrogate albumin adducts was more evident in adults than in children exposed to airborne pollutants and ETS [51,53]. Conversely, SCE and MN revealed a higher DNA damage in children than adults exposed to chemical agents [26,27] and arsenic in drinking water [24].

3.12. Comparison of biomarkers level in mothers and newborns (transplacental exposure)

The mean levels of biomarkers of genetic damage assessed in newborns and their mothers and the maternal/fetal MRs are shown in Table 12 by assay and exposure type. Among the studies reviewed, maternal cigarette smoking during pregnancy was the most frequently investigated source of exposure to the fetus (SMKU), the effect being reported in 13 studies [30,31,33–35,37,41,44–49,52,57], five measuring SCE frequency, five hemoglobin and two DNA adducts, and one each of the following: albumin adducts, *HPRT* and DNA damage (Comet assay).

Table 11

Levels of selected biomarkers detected in adults and children by type of exposure and ratio (MR) of the mean level detected in adults relative to children

Biomarker	Exposure type ^a	Exposure definition/source	Adults/children comparison				First author, year	
			Group	Sample Size	Mean \pm S.E. ^b	MR		
Biological specimen		Referents definition						
SCE	SAW	Residents downwind of chemical disposal site (non-smokers only)	Exposed	Adults = 27	10.04 \pm 0.59 ^{nr}	0.86	Lakhanisky, 1993	
Peripheral blood	Belgium	Residents in semi rural area (non-smokers only)	Referent	Children = 22	11.73 \pm 0.44	1	(Laurent, 1993)	
				Adults = 17	6.96 \pm 0.16 ^{nr}	1.23		
				Children = 11	5.64 \pm 0.25	1		
Albumin adducts Peripheral blood	ETS USA	Smokers in household	Exposed	Mothers = 32	0.49 \pm 0.08 ^{nr}	2.72	Crawford, 1994	
				Children = 31	0.18 \pm 0.04	1		
	No smokers in household	Referent	Mothers = 24	0.31 \pm 0.08 ^{nr}	2.07			
			Children = 23	0.15 \pm 0.02	1			
Comet Nasal epithelium	UAP Mexico	Airborne pollutants (Metropolitan Mexico City) Low polluted coastal town (Manzanillo)	Exposed	Adults = 69	76.8 \pm 4.5 ^{c, nr}	1.22	Calderón- Garcidueñas, 1996	
				Referent	Children = 16	63 \pm 13		1
					Adults = 18	14.75 \pm 2.06 ^{nr}		0.85
MN Peripheral blood	SAW Argentina	High level of arsenic in drinking water	Exposed	Adults = 12	41.0 \pm 4.9 ^{nr}	1.17	Dulout, 1996	
				Children = 10	35.0 \pm 4.6	1		
		Low levels of arsenic in drinking water	Referent	Adults = 10	8.5 \pm 3.4 ^{nr}	1.52		
				Children = 12	5.6 \pm 1.6	1		

The superscript "nr" represents statistical comparison not reported.

^a See footnote Table 3.^b Mean values \pm standard error (S.E.).^c Mean value \pm S.E. computed from collapsed subgroups, assuming independency of subgroups and weighted for the variance of each subgroup.

Table 12

Levels of biomarkers detected in mothers' peripheral blood and their newborns' cord blood and maternal/fetal ratio (MR) by type of assay and exposure

Biomarker	Exposure type ^a	Exposure definition/source	Mothers/newborns comparison				
			Assay type/units	Referents definition	Group	Sample size	Mean \pm S.E. ^b
SCE SCEs/cell	SMKU (USA)	Mothers smoking 8–40 cigarettes per day	Exposed	Mothers = 21 Newborns = 21	13.5 \pm 0.56 ^{nr} 9.7 \pm 0.38	1.39 1	Lundgren, 1987
		Non-smoking mothers	Referent	Mothers = 22 Newborns = 22	11.1 \pm 0.35 ^{nr} 9.6 \pm 0.56	1.16 1	
SCE SCEs/cell	SMKU (Finland)	Mothers smoking	Exposed	Mothers = 17 Newborns = 17	9.0 \pm 0.9 ^{nr} 6.1 \pm 0.5	1.48 1	Sorsa, 1988
		Exposed to ETS (smokers in household)	Referent	Mothers = 7 Newborns = 7	8.4 \pm 1.0 ^{nr} 5.9 \pm 0.8	1.42 1	
		Not exposed to ETS	Referent	Mothers = 18 Newborns = 19	7.9 \pm 0.9 ^{nr} 5.9 \pm 0.5	1.34 1	
SCE SCEs/cell	SMKU (Turkey)	Mothers smoking \geq 1 cigarettes per day	Exposed	Mothers = 21 Newborns = 21	7.31 \pm 1.40* 5.2 \pm 0.31	1.41 1	Şardaş, 1995
		Exposed to ETS (smokers in household)	Referent	Mothers = 8 Newborns = 8	5.36 \pm 0.13* 4.16 \pm 0.20	1.29 1	
		Not exposed to ETS	Referent	Mothers = 10 Newborns = 10	5.06 \pm 0.20* 3.83 \pm 0.14	1.32 1	
SCE SCEs/cell	SMKU (Italy)	Mothers smoking \geq 5 cigarettes per day	Exposed	Mothers = 10 Newborns = 10	8.13 \pm 1.13* 5.74 \pm 0.76	1.42 1	Ardito, 1980
		Non-smoking mothers	Referent	Mothers = 10 Newborns = 10	8.14 \pm 1.07* 5.94 \pm 0.73	1.37 1	
SCE SCEs/cell	SmKU (Australia)	Mothers smoking \geq 5 cigarettes per day	Exposed	Mothers = 23 Newborns = 25	11.88 \pm 0.41 ^{nr} 9.01 \pm 0.28	1.32 1	Seshadri, 1982
		Non-smoking mothers	Referent	Mothers = 30 Newborns = 30	11.35 \pm 0.40 ^{nr} 8.95 \pm 0.37	1.27 1	
Hemoglobin adducts HOEtVal (pmol/g Hb)	SMKU (Portugal)	Mothers smoking 15 cigarettes per day	Exposed	Mothers = 13 Newborns = 13	361 \pm 29.68 ^{nr} 147 \pm 18.86	2.46 1	Tavares, 1994
		Non-smoking mothers	Referent	Mothers = 10 Newborns = 10	63 \pm 6.32 ^{nr} 42 \pm 5.69	1.50 1	
Hemoglobin adducts HOEtVal (pmol/g Hb)	SMKU (Denmark)	Mothers smoking	Exposed	Mothers = 20 Newborns = 20	150.9 ^{nr} 55.2	2.73 1	Farmer, 1996
		Non-smoking mothers	Referent	Mothers = 29 Newborns = 29	30.3 ^{nr} 7.3	4.15 1	
Hemoglobin adducts 4-ABP (pg/g Hb)	SMKU (USA)	Mothers smoking >40 cigarettes per day	Exposed	Mothers = 21 Newborns = 21	488 \pm 37.97 ^{nr} 244 \pm 19.86	2.00 1	Pinorini-Godly, 1996
		Non-smoking mothers	Referent	Mothers = 21 Newborns = 21	29.6 \pm 3.54 ^{nr} 14.0 \pm 1.42	2.11 1	

Hemoglobin adducts 4-ABP (pg/g Hb)	SMKU (USA)	Mothers smoking >40 cigarettes per day	Exposed	Mothers = 20	633 ± 19.7 ^{nr}	1.98	Myers, 1996
				Newborns = 20	319 ± 11.3	1	
		Mothers smoking	Exposed	Mothers = 74	367 ± 22.4 ^{nr}	1.99	
				Newborns = 74	184 ± 11.6	1	
		Non-smoking mothers	Referent	Mothers = 74	18.3 ± 1.5 ^{nr}	2.06	
				Newborns = 74	8.88 ± 0.67	1	
Hemoglobin adducts 4-ABP (pg/g Hb)	SMKU (USA)	Mothers smoking	Exposed	Mothers = 14	183 ± 28.86 ^{nr}	1.99	Coghlin, 1991
				Newborns = 14	92 ± 14.43	1	
		Non-smoking mothers	Referent	Mothers = 38	22 ± 2.14 ^{nr}	1.29	
				Newborns = 38	17 ± 2.11	1	
Albumin adducts BaP (ELISA)	SMKU (Denmark)	Mothers smoking	Exposed	Mothers = 21	Graphically depicted data	1.37 ^d	Autrup, 1995
				Newborns = 21		1	
		Non-smoking mothers	Referent	Mothers = 107	Graphically depicted data	1.25 ^d	
				Children = 107		1	
DNA adducts PAH ELISA (<i>n</i> /10 ⁸ nucleotides)(Poland)	SMKU	Mothers smoking up to delivery	Exposed	Mothers = 12	12.4 ± 4.5 ^{nr}	2.03	Whyatt, 1998
				Newborn = 7	6.1 ± 3.7	1	
		Mothers smoking during pregnancy (including ex smokers)	Exposed	Mothers = 31	6.6 ± 1.2 ^{c.nr}	1.12	
				Newborns = 26	5.9 ± 1.83	1	
		Non-smoking mothers	Referents	Mothers = 26	6.9 ± 1.7 ^{nr}	1.23	
				Newborns = 32	5.6 ± 1.2	1	
DNA adducts PAH ELISA (<i>n</i> /10 ⁸ nucleotides)(Poland)	SMKU	Mothers smoking	Exposed	Mothers = 10	12.9 ± 5.53	2.80	Whyatt, 2001
				Newborns = 10	4.6 ± 2.66	1	
		Non-smoking mothers	Referents	Mothers = 70	5.1 ± 1.12	0.63	
				Newborns = 70	8.1 ± 1.12	1	
DNA adducts PAH ³² P-postlabelling (<i>n</i> /10 ⁸ nucleotides)	SMKU (Poland)	Mothers smoking	Exposed	Mothers = 11	12.0 ± 1.27	0.82	Whyatt, 2001
				Newborns = 11	14.7 ± 4.13	1	
		Non-smoking mothers	Referents	Mothers = 79	15.0 ± 1.83 [*]	0.88	
				Newborns = 79	17.1 ± 1.27	1	
DNA adducts PAH ELISA (<i>n</i> /10 ⁸ nucleotides)(Poland)	ETSU	Non-smoking mothers exposed to ETS	Exposed	Mothers = 16	9.1 ± 2.6 ^{nr}	1.49	Whyatt, 1998
				Newborns = 17	6.1 ± 1.7	1	
		Non-smoking mothers not exposed to ETS	Referents	Mothers = 10	3.3 ± 1.0 ^{nr}	0.67	
				Newborns = 15	4.9 ± 1.8	1	
DNA adducts BaP HPLC-fluores. (<i>n</i> /10 ⁸ nucleotides)	ETSU (USA)	Non-smoking mothers exposed to ETS	Exposed	Mothers = 100	0.23 ± 0.01	0.96	Perera, 2004
				Newborns = 165	0.24 ± 0.01	1	
		Non-smoking mothers not exposed to ETS	Referent	Mothers = 38	0.21 ± 0.02	0.88	
				Newborns = 38	0.24 ± 0.02	1	
Comet Assay % DNA in tail	SMKU (Czech Republic)	Mothers smoking	Exposed	Mothers = 134	5.00 ± 0.11 ^{nr}	0.94	Šrám, 1998
				Newborns = 134	5.31 ± 0.12	1	
		Non-smoking mothers	Referent	Mothers = 213	5.15 ± 0.09 ^{nr}	0.94	
				Newborns = 213	5.45 ± 0.09	1	

Table 12 (Continued)

Biomarker	Exposure type ^a	Exposure definition/source	Mothers/newborns comparison				
			Assay type/units	Referents definition	Group	Sample size	Mean \pm S.E. ^b
Comet Assay % DNA in tail	UAP (Czech Republic)	Air pollution from industry and residential heating (Teplice)	Exposed	Mothers = 211 Newborns = 211	5.05 \pm 0.09 ^{nr} 5.26 \pm 0.9	0.96 1	Šrám, 1998
		Residents in agricultural districts (Prachatice)	Referent	Mothers = 137 Newborns = 137	5.15 \pm 0.12 ^{nr} 5.59 \pm 0.12	0.92 1	
CA % Aberrant cells	UAP (Czech Republic)	Air pollution from industry and residential heating (Teplice)	Exposed	Mothers = 54 Newborns = 86	1.22 ^{nr} 0.89	1.37 1	Šrám, 1999
		Residents in agricultural districts (Prachatice)	Referent	Mothers = 29 Newborns = 20	1.05 ^{nr} 1.00 ^f	1.05 1	
HPRT Autoradiographic (Vf/10 ⁶)	SMKU (USA)	Mothers smoking \geq 4 cigarettes per day	Exposed	Mothers = 5 Newborns = 10	3.08 \pm 0.55 2.17 \pm 0.24	1.42 1	Ammenheuser, 1994
		Non-smoking mothers, not exposed to ETS	Referent	Mothers = 5 Newborns = 9	1.07 \pm 0.17 ^{nr} 0.77 \pm 0.13	1.39 1	

The superscript “nr” represents statistical comparison not reported.

^a See footnote Table 3.

^b Mean values \pm S.E. (when reported in the original paper).

^c MR, mean levels in exposed mothers relative to their newborns and referent mothers relative to their newborns.

^d MR as reported in the original paper.

^e Mean value \pm S.E. computed from collapsed subgroups, assuming their independency and weighted for the variance of each subgroup.

^f Statistically significant difference as indicated in the original paper.

SCE frequency was consistently higher in smoking mothers than in their newborns in all studies, with maternal/fetal MRs ranging between 1.32 and 1.48 (Table 12). In referent subjects (i.e., non-smoking mothers and newborns), MRs of SCE ranged from 1.16 to 1.37. The maternal/fetal MRs were always higher in exposed than in referents subjects. Hemoglobin adduct levels were always higher in mothers with maternal/fetal MRs ranging between 1.98 and 2.73 in smoking and 1.29 and 4.15 in non-smoking mothers during pregnancy [44–48]. The maternal/fetal MR for hemoglobin adducts was higher in referents than in exposed subjects in two studies [46,48].

Albumin adducts were higher in smoking as well as in non-smoking mothers during pregnancy when compared to their newborns (MR = 1.37 and 1.25) [49].

For DNA adducts, the MR ranged between 0.82 and 2.80 in exposed to cigarette smoke compounds, and between 0.63 and 1.23 in referent subjects. The difference between DNA damage in mothers and newborns appeared to be assay dependent, with MRs > 1 in smoking exposed pairs when DNA adducts were measured by the enzyme-linked immunoassay (ELISA) [37,41], and with MRs < 1 when the 32P-postlabelling assay was used [41]. DNA damage assessed by the Comet assay was lower in mothers than in newborns (MR = 0.94), regardless of the mothers' smoking status [52]. Conversely, *HPRT* frequency was 40% higher in maternal tissue regardless of the smoking status (MR = 1.42 and 1.39, in exposed and referents, respectively) [57].

The effect of intrauterine exposure to ETS on DNA adducts was investigated in two studies conducted in Poland and in the USA [37,39]. Higher levels of DNA adducts were detected in Polish non-smoking mothers exposed to ETS (MR = 1.49), while non-smoking mothers not exposed to ETS had lower levels than their newborns (MR = 0.67) [37]. Higher DNA adduct levels were measured in Dominican and African-American newborns exposed in utero to ETS (MR = 0.96), and in newborns not exposed to ETS than in their mothers (MR = 0.88) [39].

The effect of urban air pollution on the level of CA and of DNA damage (Comet assay) was investigated in newborn–mother populations from selected areas

of the Czech Republic [13,52]. CA mean levels were increased in mothers exposed to air pollutants compared to their newborns (MR = 1.37) but not in referent mothers (MR = 1.05). The maternal/fetal ratio computed in Czech populations was 0.96 and 0.92 in exposed to airborne pollutants and in referents, respectively, indicating lower DNA damage levels measured by Comet assay in mothers than newborns.

4. Discussion

4.1. Exposure-biomarker associations

The findings of the present review suggest that field studies conducted in children exposed to environmental pollutants may greatly benefit from the inclusion of biomarkers of genetic damage as study end-points. In fact, among the most frequently used cytogenetic biomarkers, CA and MN were consistently increased in children exposed to environmental pollutants regardless of the type of exposure. SCE appeared to be a less sensitive early biological effect marker capable of detecting only, and inconsistently, exposures to air, soil and drinking water contaminants and fetal exposure to smoking compounds. Postnatal exposure to ETS was associated with higher levels of SCE in a single study. Hemoglobin and DNA adducts were consistently increased in children exposed to airborne urban pollutants and ETS, and in newborns exposed in utero to cigarette smoke as well as ETS. Albumin adducts were increased in children exposed to ETS in their house, in newborns delivered by smoking mothers, and also in newborns delivered by urban-resident non-smoking mothers compared to rural residents. DNA damage assessed by the Comet assay was increased in children exposed to heavy urban air pollutants in Mexico City but not in Teplice, and in children exposed to environmental pollution from mining, smelting and waste incineration activities. Higher *HPRT* mutant frequencies were reported in newborns whose mothers smoked or were exposed to ETS during pregnancy compared to newborns delivered by mothers not exposed to genotoxic compounds contained in mainstream smoke and ETS.

4.2. Meta-analyses

The exposure-biomarker specific ratios estimated through meta-analysis confirmed the associations between hemoglobin adducts and postnatal exposure to ETS (meta-MR = 1.3) and transplacental exposure to smoking compounds (meta-MR = 6.7). Although hemoglobin adducts are a surrogate measure of DNA adduction, the detected association between postnatal exposure to ETS suggests a 30% increased level of biologically effective dose, which is in line with the increased lung cancer risk reported in adults who have never smoked and were exposed to ETS during childhood [62]. The almost seven-fold increased level of hemoglobin adducts (meta-MR = 6.7) that was observed in newborns exposed to cigarette smoke in utero compared to non-exposed newborns is in agreement with the epidemiological evidence of smoking-related cancer risks, with lung cancer occurring 7–15 times more frequently in smokers than in non-smokers [63]. The observed correspondences, although impressive, should be interpreted with caution, keeping in mind that the computed meta-MRs may suffer from the limited number of studies that were suitable for meta-analysis, the limited sample size, the potential biases in single studies, and the possibility of a preferential publication of positive results (publication bias) [64]. The latter would have lead to an overestimated evidence of the exposure-biomarkers association. However, a search of unpublished findings (including governmental reports and dissertations) and book chapters that was conducted within the CHILDRENGENONETWORK failed to identify studies not retrieved through MedLine search and manual inspection of the references cited in relevant papers, suggesting no evidence of under reporting of negative findings.

4.3. Comparison of biomarker levels in children and adults

Measuring biomarkers of genetic damage in adults and children or in mothers and newborns exposed to environmental pollutants may be considered a quasi-experimental design suitable to investigate the effects of exposure to the same cocktail of agents and evaluate age-related susceptibility. The underlying assumption is that, under uncontrolled experimental conditions,

children and adults are exposed to the same levels of genotoxic agents. This can be true, to some extent, for fetal exposures, where the mother's lifestyle during pregnancy mostly determines intrauterine exposure. However, the placenta efficiently and selectively acts as a barrier against the transfer of environmental toxicants from the mother to the fetus. For example, less than 10% of the mother's intake of PAH crosses the placenta [10,11] and reaches the fetus. For exposures occurring during postnatal developmental periods, e.g., childhood and adolescence, where the assumption of "equal exposure" is based on the identification of children and adults sharing common exposures, it is essential to account for lifestyle and other individual factors that may differentially affect exposure levels. Keeping all this in mind, we addressed the issue of an increased susceptibility to genotoxic exposures of children and newborns by computing biomarker-exposure specific maternal/fetal and adults/children mean ratios (MRs) in exposed and referent subjects. The adults/children comparison must take into account the highest baseline level detected in adults for CA, SCE and MN [65,66], leading to adults/children MRs > 1. The age effect was confirmed in a pooled analysis of children aged 0–19 years, showing a very low MN baseline level at birth (3.27‰ binucleated cells), increasing by 66% in children aged 1–4 years (5.43‰ cells) [67]. Moreover, although some biomarkers are known to be less influenced by age, e.g., DNA adducts [68], biological differences between newborns and adults would also result in maternal/fetal MRs > 1. This is the case for the formation of HOEtVal globin adducts, because fetal hemoglobin (HbF), which is a major form of haemoglobin present during uterine life and up to 3 months following birth, contains only two Val-N-terminal α -polypeptide chains in contrast to the predominant hemoglobin (HbA) found in adults, which contains four Val-N-terminal polypeptide chains [69]. However, if children are more susceptible than adults, and if a positive exposure-biomarker association exists, the biomarker baseline level is expected to increase more in exposed children than in exposed adults, and the adults/children MR will be smaller in exposed than referent subjects. Indeed, higher levels of genotoxic damage (MN and DNA single strand breaks) have been reported in fetal than in maternal tissue of rodents following transplacental exposure to

benzo(a)pyrene [70–72]. Of the four studies included in the present review reporting data for children and adults, the adults/children MRs was lower in exposed than in referent subjects for MN and SCE measured in individuals exposed to arsenic in drinking water and to environmental pollutants released from a chemical dump, respectively. Although these findings are suggestive of a higher susceptibility of children than adults, the limited number of studies available (one for each biomarker), preclude any meaningful conclusion.

4.4. Comparison of biomarker levels in mothers and newborns (transplacental exposure)

Among the studies conducted in mothers and newborns, consistently higher maternal/fetal MRs were detected for SCE in exposed than in referent subjects. Maternal/fetal MRs ranged between 1.32–1.48 and 1.16–1.42 in exposed and in referent subjects. These findings do not support the evidence of a higher susceptibility of children than adults to environmental genotoxic compounds. However, SCE frequency was found to be a poorly sensitive and inconsistent marker of early effect, with a few, sporadic exceptions.

Hemoglobin adducts' maternal/fetal MRs were slightly lower in cigarette smoke exposed than in referent subjects in two studies, clearly lower in one, and higher in two. The overall evidence provided by this marker of biologically effective dose is supportive of a high susceptibility of children, considering that the studies reporting clearly higher maternal/fetal MRs in exposed than in referent subjects were based on a limited number of mother–newborn pairs exposed to cigarette smoke.

PAH ³²P-postlabelling- and BaP-HPLC-DNA adduct levels were lower in smokers, ETS exposed and referent mothers than in newborns, with maternal/fetal MRs < 1, suggesting per se a higher susceptibility of the fetus to specific environmental agents. Less consistent findings were reported by the enzyme-linked immunoassay, with higher maternal than fetal levels (MRs > 1) measured in smoking and ETS exposed pairs and higher fetal levels (MRs < 1) measured in non-smoking as well ETS unexposed maternal/fetal pairs. This biomarker of the biologically effective dose supports, to some extent, the increased susceptibility of the fetus to genotoxic agents, specifically PAH.

The level of DNA damage assessed by the Comet assay was slightly higher in fetal than maternal tissues (MR < 1) in subjects exposed to cigarette smoke as well to urban air pollutants, supporting children's increased susceptibility to environmental genotoxic agents. A single study reporting the effect of smoking in utero on *HPRT* mutant frequency measured in a very small number of subjects, showed increased maternal/fetal ratio (MRs > 1).

5. Conclusions

The present review shows that biomarkers of genetic damage, including CA and MN, DNA adducts and DNA-surrogate hemoglobin and albumin adducts are capable of detecting early biological effects in children and newborns exposed to environmental pollutants. These biomarkers were consistently increased for postnatal exposures to urban air pollutants and ETS and transplacental exposure to chemicals contained in mainstream cigarettes smoke (i.e., smoking mothers) as well as in second-hand cigarettes smoke (ETS). Hemoglobin adducts levels were 30% higher (meta-MR = 1.38), in children exposed to ETS in utero compared to non-exposed, and almost seven times higher (meta-MR = 6.65) in newborns delivered by mothers who smoked during pregnancy compared to non-smoking mothers. Active smoking, exposure to ETS or to urban air pollutants during pregnancy were associated with higher aromatics- and PAH-DNA adduct levels and DNA damage (Comet assay) measured in fetal than in maternal blood, providing evidence for a higher susceptibility to DNA damaging chemical compounds of children than adults. PAHs have been shown to be transplacental carcinogens in rodent bioassays [73], resulting in a higher cancer incidence following prenatal and perinatal exposure compared to exposure occurring later in life [74–76]. Increased PAH-DNA adduct levels, although DNA adduction is not sufficient for tumorigenesis, and more generally, increased levels of biomarkers of genetic damage in children, could have important implications for adverse health effects [77–79]. Indeed, an increased frequency of CA has been reported to predict cancer occurrence in healthy adults [80] and, if this link holds for children, biomonitoring programs would greatly

benefit from the inclusion of selected exposure specific biomarkers. The detection of increased biomarker levels could be used as sound, biologically based evidence for the identification of intervention priorities in environmental health aimed at protecting children's health.

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