

PRENATAL, EARLY LIFE, AND CHILDHOOD EXPOSURE TO GENOTOXICANTS IN THE LIVING ENVIRONMENT

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Health disorders and diseases related to environmental exposure in children such as cancer and immunologic disturbances (asthma, allergies) are on the rise. However, complex transplacental and prepubertal genotoxicology is given very limited consideration, even though intrauterine development and early childhood may be critical for elucidating the cancer aetiology. The foetus is transplacentally exposed to contaminants in food and environment such as various chemicals, drugs, radiochemically contaminated water and air. Target organs of xenobiotic action may differ between the mother and the foetus due to specific stage of developmental physiology and enzyme distribution. This in turn may lead to different levels of clastogenic and aneugenic metabolites of the same xenobiotic in the mother and the foetus. Adult's protective behaviour is not sufficient to isolate children from radioisotopes, pesticides, toxic metals and metalloids, environmental tobacco smoke, endocrine disrupting chemicals, and various food contaminants, which are just a part of the stressors present in a polluted environment. In order to improve legislation related to foetus and child exposure to genotoxic and possibly carcinogenic agents, oncologists, paediatricians, environmental health specialists, and genotoxicologists should work together much more closely to make a more effective use of accumulated scientific data, with the final aim to lower cancer incidence and mortality.

KEY WORDS: *child genotoxicology, environmental exposure, genome damage, transplacental genotoxicology, xenobiotics*

Health disorders and diseases related to environmental exposure in children such as cancer and immunologic disturbances (asthma, allergy) are on a constant rise (1-3). The increased risk of cancer could be the result of *in utero* and early life exposure (4). Carcinogenesis could start during the specifically vulnerable period of foetal development as a consequence of maternal exposure to food and environmental mixture of pollutants (5, 6). The complex interaction between the environment and a living organism is modified by processes of growth, development, adaptation, and aging. Challenges to developmental homeostasis may burden adulthood

with different health risks and deviations in processes of ageing. Radiation and chemical carcinogens can damage the genome through direct interaction with DNA, formation of free radicals, disturbances of lipid peroxidation, and hypomethylation (7-9). As it seems that almost all cancers are oestrogen-related, evaluation of carcinogens demands a new approach (10-12). Oestrogen-like activity has been described for known or suspected genotoxic agents such as metals, heterocyclic amines, some pesticides, and dioxins (13-16). Cotinine (a nicotine metabolite) and some fungicides are, in contrary, aromatase inhibitors (17, 18). Oestrogen also has a significant role in

organogenesis and in maturation during the pubertal period (19). In addition, hormone levels can cause specific radiochemical sensitivity (20).

Genome damage in the foetus is a result of a complex interaction between maternal and foetal metabolism, development stage of the foetus, pregnancy-related bioaccumulation of metabolites and detoxification capacity of the foetus and the mother (including endometrium) (21). Interaction between the xenobiotics and their metabolites even at low doses may be synergistic and long-term. Furthermore, production of clastogenic or aneugenic metabolites of the same xenobiotic can differ between the first and the third trimester of pregnancy. Drugs like paracetamol, 5-nitrofurantoin, or fluconazole, which are frequently prescribed in paediatric practice, are potential transplacental and postnatal genotoxins as is found in the latest investigations in young animals (22, 23).

Human population, is exposed to hazardous biological and chemical agents through air, water, or food (Figure 1). The foetus is exposed to (radio)chemical genotoxins from the occupational and living environment of the mother and father and for children it is water, air at home and school (indoor), urban or rural open air microenvironment and food. The effects of exposure to lead, polychlorinated biphenyls (PCBs), radioisotopes, environmental tobacco smoke, mercury, endocrine disrupters,

pesticides, and food contaminants are often combined with drugs (including recreational ones) and stress at school, in the family (such as divorce) or society (such as war), infection and malnutrition.

All too often environmental risks are recognised when it is too late, that is, through epidemiological studies, when health consequences are already there. Genotoxicological methods in properly modelled studies, can identify genome damage and health risk before the first clinical symptoms. The most informative result is produced by measuring the final biological effect, which summarises all synergistic and antagonistic mechanisms of xenobiotics. Molecular epidemiology uses biomarkers that can measure the concentration of xenobiotics and their metabolites (biomarkers of exposure), individual's susceptibility (biomarkers of susceptibility), and early biological effects through molecular and cellular changes (biomarkers of effect). These biomarkers of effect include chromosome aberration (CA), micronuclei (MN), and sister chromatid exchange (SCE), which are used in biomonitoring. CA and MN correlate with increased cancer risk (24, 25). Another valuable tool, the comet assay, has been used to measure genotoxic effects and individual susceptibility in humans. Despite its advantages such as great sensitivity and small sample size required, the comet assay has not yet been standardised with a protocol and a quality control programme (26, 27).

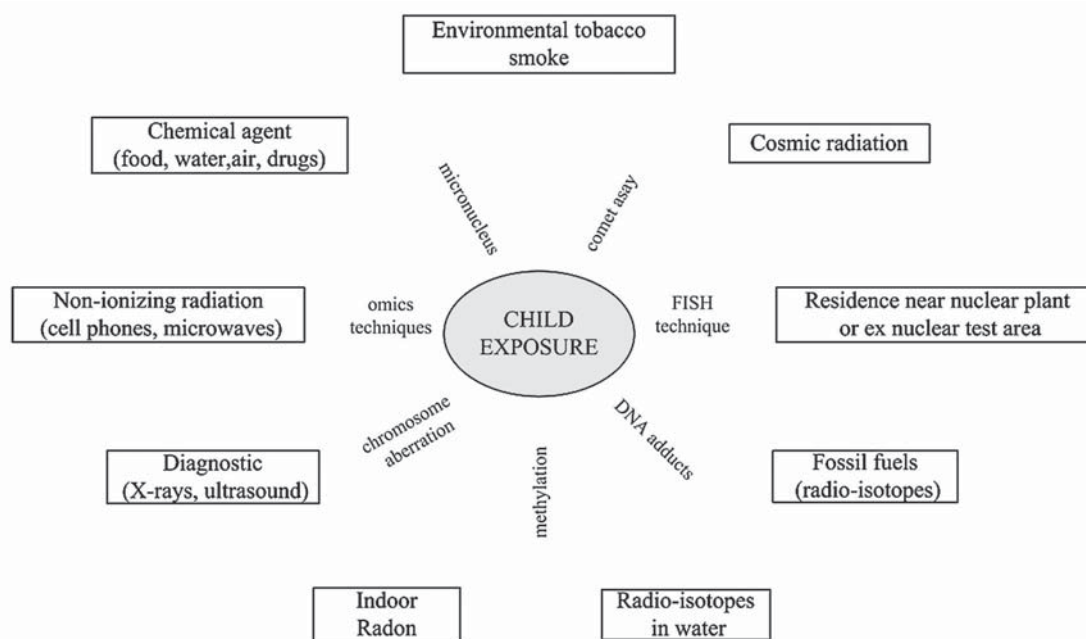


Figure 1 Children's exposure to physical and chemical agents and applied biomarkers of susceptibility, exposure, and effect

Infants and children are generally omitted from large-scale biomonitoring studies because of ethical reasons. Literature search showed that applied genotoxicological methods are sensitive to genome damage in children caused by xenobiotics like those from environmental tobacco smoke and air pollution (28, 29). In a meta-analysis by Neri et al. (28), CA and MN were increased in children exposed to industrial pollutants, chemical waste, polluted water, indoor radon, and environmental tobacco smoke. Although used in only a few studies, SCE also deviated from control values in children who lived downwind of a chemical disposal site (30). Several studies of child exposure to chemical agents used the following biomarkers: DNA, haemoglobin and protein adducts, comet assay, and gene mutation assay (31-35). They suggest specific susceptibility of the foetus and child and call for further search for the most sensitive methods for certain types of environmental (radio)chemicals (30, 36).

This paper gives an overview of child exposure to genotoxicants in food, drugs, water, air, including ionising, and non-ionizing radiation.

FOOD

Genotoxic agents are detectable in food at levels which vary depending on the type of food and preparation method. For instance, baking and broiling causes formation of acrylamide, heterocyclic amines, and benzo(a)pyrene at different levels (37-39). However, contrary to professional and environmental exposure where one can have only small influence, nutrition although defined by socioeconomic and demographic status, can be improved. Changes in dietary habits, size of meal, and cooking method can also reduce intake of contaminants. Recommendations for optimal and balanced diet are of particular importance for pregnant women in order to reduce foetal exposure. Deficiency in micronutrients (minerals, vitamins) in maternal nutrition could cause adverse health effects on foetal development (40, 41). So far there is not much scientific evidence confirming newborn genome damage due to maternal exposure to genotoxic chemicals present in food during pregnancy. Ross et al. report increased risk of infant leukaemia if the mother is exposed to drugs or natural substances in food that are inhibiting DNA topoisomerase II (42). One of the most investigated

dietary transplacental toxicants in humans is ethanol (EtOH). Metabolised with higher efficiency in mothers, EtOH may cause severe biological effects in the foetus due to low foetal clearance capacity (43). Chronic exposure to aflatoxin, a mycotoxin present in food, may have carcinogenic effects (44). Lipoxygenase activity caused by aflatoxin B-1 is significantly higher in prenatal than in adult liver tissue, while detoxification by epoxide hydrolase is half as effective as in adults (45).

Prenatal exposure to PCBs could adversely affect child's neurological and cognitive development (46). Karttunen et al. (47) have shown that maternal exposure to benzo(a)pyrene can lead to placental biotransformation which produces reactive metabolites able to form stable BaP-DNA adducts in foetus. Maternal high fish consumption during pregnancy could lead to prenatal mercury and methylmercury exposure leading to foetal death or neurodevelopmental delays (48). However, there are no data on heavy-metal-related genome damage and health risk in newborns or on any late genotoxic effects during childhood.

DRUGS

Developmental toxicants became an issue in the 1960s when the use of thalidomide was shown to have deleterious effects on the foetus development (49). Since then, a number of medications have been prescribed during pregnancy but less than 10 % of drugs have been investigated for teratogenic effects (50). Studies have shown that the placental barrier is permeable to drugs and their metabolites (51), but there are few of them investigating and evidencing genome damage in newborns. We have recently reported new *in vivo* findings of MN induction in newborns of mothers taking antiepileptic drugs during pregnancy (52). Witt et al. (53) reported a tenfold increase in micronucleated reticulocyte frequency in infants exposed to antiretroviral therapy *in utero*.

WATER

The usual genotoxicants in water are nitrate, arsenic, nickel, cadmium, and asbestos (54) and children exposed to them have shown genome damage (55, 56). Unlike tap water, bottled water

originating from ground water can contain ^{226}Ra , ^{228}Ra , ^{210}Pb , and ^{210}Po and display various levels of radioactivity (57). Its consumption has increased in order to avoid chemical contaminants in tap water, but also due to changes in eating habits and lifestyle. Several studies of bottled water samples in Europe have found beta radioactivity going up to 4.6 Bq L^{-1} and alpha radioactivity up to 1.75 Bq L^{-1} , which is well above the reference limits (1.0 Bq L^{-1} and 0.1 Bq L^{-1} , respectively) (59-60). World Health Organization (WHO) (61) recommends that the annual effective dose from water consumption does not exceed 5 % of the average effective dose from natural sources (2.4 mSv), that is, 0.1 mSv . As radon follows the metabolic pathway of calcium, its incorporation into a child's skeleton poses a significant health risk (62). Due to age-dependent development of the gastrointestinal system in children, the highest absorption of radon is in newborns and children between 13 and 17 years of age (63, 64). In addition, newborns and children seem to drink more water than adults (65). Undernourished infants may receive doses of up to $0.28 \text{ mSv year}^{-1}$ if their diet is exclusively prepared with mineral water with elevated radon concentrations. According to Bronzović et al. (66) ^{226}Ra body activity is significantly higher in people exposed to high doses during childhood than those exposed at adult age. Accumulation of radon in bones during puberty may be related to specific hormonal activity of testosterone and oestrogen (67). The mechanism of ^{90}Sr bioaccumulation in the bone is similar to ^{226}Ra , but according to Tolstykh et al. (68) its bone affinity is even greater, as shown in a population overexposed to ^{90}Sr near the Mayak plutonium production complex in Russia. These authors find that the most critical period for girls is just before menarche and for boys 2 to 3 years before the formation of the secondary sex features. Consequently, adults who were exposed to ^{90}Sr during the childhood have a significantly higher frequency of translocations than young adults exposed at the age of 20 years or older (69).

In summary, water involves exposure to a complex mixture of radiochemicals. To understand their mechanisms of action one needs to be aware of several pathways including knowledge on specific early age or later hormonal level related bioaccumulation.

AIR

Children spend most of their time at home or at (pre)school, where they may be exposed to

formaldehyde. This can lead to higher CA frequency (70, 71). Exposure to secondary tobacco smoke leads to deviations in all genotoxic biomarkers from control values for all age groups, from newborns to adolescents (28, 72). Hansen et al. (73) have shown that carcinogens from tobacco smoke pass through the placenta to foetal tissues and metabolise to DNA-damaging agents. They have also found that newborns of mothers who smoked and drank alcohol during pregnancy had higher translocation frequencies. Cadmium, lead, and arsenic, from tobacco smoke have 10 times higher absorption than from food or water (74). In a study by Godschalk et al. (75), foetal exposure to heavy metals such as cadmium correlated positively with the number of HPRT-variants per adduct in cord blood. The authors suggest that by inhibiting DNA repair, cadmium may enhance the genotoxic effect of other carcinogens.

Radon gas is the second leading cause of lung cancer after smoking. Apart from background radon radiation, indoor exposure is usually related to natural radioactivity from fly ash, alum shale, and phosphogypsum used in building materials (76). Stoulos et al. found increased MN and CA frequency in children exposed to high levels of indoor radon (77).

Exposure to outdoor airborne pollutants, including chemicals from suburban smelters or residential heating systems can cause genome damage in children (78-80). Pedersen et al. (81) found that maternal exposure to traffic-related air pollution in urban environment resulted in increased bulky DNA adducts and MN frequency in cord blood (81). A life close to mining sites may be the source of severe genome damage in children caused by arsenic or lead (82) but it may also be contaminated by radioisotopes especially if mines are followed by the nucleo-chemical industry (83).

IONISING RADIATION

Most of the data available on children's genome damage caused by ionising radiation are related to accidental overexposure in Chernobyl (Ukraine), the Techa River (Russia), and Goiânia (Brasil) or are related to medical therapy (28,29). Although pregnant women are usually isolated from jobs which could jeopardise normal development of the foetus, the first trimester of pregnancy can go unnoticed, and the foetus may be exposed to increased risk. In hospitals where

women are occupationally exposed to radioisotopes (iodine, chromium, thallium, technetium, thorium) the miscarriage incidence is significantly higher than in women occupationally exposed to X-rays (36). It seems that health effects of radioisotopes applied in diagnostics are not limited to their own radioactivity but may also be owed to contamination with lead, tin, or nickel due to the technology of production, which act as heavy metals or xenoestrogens (36).

Epidemiological studies of parental exposure to ionising radiation and cancer risk in children have been limited to post-Chernobyl accident biomonitoring of the so called *liquidators*, who were removing radioactive material and of the Chernobyl plant workers (84, 85). The health effects of nuclear power plants on nearby residents have not duly been investigated; this is particularly true for genome damage in children. Epidemiological studies showing clusters of leukaemia in these areas still need to be confirmed (86-89). Available studies are focused on childhood leukaemia cases in the population living in an area of about 5 km around nuclear plants. These studies suggest that beside the health risk of children whose father work at nuclear plants (90), significant increase in leukaemia cases is also present in the general population (91, 92). Another source of ionising radiation are diagnostic and treatment procedures in medicine (93). Kleinerman (94) found that child exposure to radiation sources used for diagnostic imaging increased the risk of cancer and was greatest for those exposed early in life (94). Doodly et al. (95) reported increased risk of breast cancer among women with scoliosis after multiple diagnostic X-ray examinations during childhood and adolescence (95).

NON-IONISING RADIATION

Human exposure to radiofrequency (RF) non-ionising radiation has increased over the last decade as a result of increased use of mobile phones, especially in children and adolescents (96). Epidemiological studies in animals and humans have revealed neurological and behavioural effects of RF radiation exposure (97, 98). However, cancer risk assessments have remained inconclusive, and there is only one study with strong scientific evidence of association between RF radiation from cell phones and wireless devices and the development of acoustic neuroma and glioma, especially in people who started using mobile phones

before the age of 20 years (99). Another multi-national case-control study (the Interphone study, 100), find no association between RF exposure from mobile phones and cancer risk. According to Shüz (101), newborns and children are more susceptible than adults since their nervous system is still developing. In addition, they will have much higher cumulative exposure than today's adults and the potential long-term health effects are still unknown. Therefore, current legislation should be based on the precautionary principle.

CONCLUSIONS AND RECOMMENDATIONS

Developmental characteristics of children as a response to their living environment are specific bioaccumulation, absorption, distribution, foetal and transitional perinatal metabolic and detoxification enzymes and kidney clearance (102). Adult values for glomerular filtration rate, maximum tubular excretory capacity, and maximum renal concentrating capacity are achieved by the age of 2 years. Foetuses and newborns up to 3 months of age have significantly different cytochrome P450 levels than adults, which results in different circulatory half-life for a number of substances (103). Therefore, paediatric pharmacology could help to better understand the mechanisms of action of environmental genotoxicants and to better assess the related risks (104). Similarly, knowledge of metabolic kinetic and chemical structure of environmental xenobiotics, can be used in research of new drugs.

Specific research is needed to investigate environmental impact mediated by complex hormonal changes during puberty. The interaction between PCBs, dibenzodioxins, phyto-oestrogens, cadmium or DDT and endogenous hormones (105, 106) has to be further investigated because of their potential to sensitise mechanisms leading to increased genome damage by other genotoxic agents from the environment (107).

In transplacental studies, knowledge of metabolism during intrauterine development should be incorporated in interpretation of results, as the same xenobiotics may cause different levels of genome damage in different foetal tissues through time.

Future research demands: pharmacokinetic studies across different developmental stages, molecular characterisation of target-binding sites, and better understanding of the synthesis and activation of

nuclear proteins (transcription factors), nucleotide pool disturbances, and impact of lipid peroxidase products (4-hydroxynonenal and malondialdehyde).

Over the last few decades, genotoxicology and developmental genotoxicology have been limited to frozen time segment insights into the mechanisms that lead to genome damage. Introduction of systems biology, a new field which develops a system-level understanding of biology (108) enables analysis of dynamics of the systems, optimal frame for interpretation of collected data on the interaction between environment and known developmental mechanisms. Recent advances have demonstrated that molecular regulatory networks can be modelled in mathematical terms. Such approach will prevent hundreds of genotoxicological results and especially results from gene and protein arrays to serve simply as a catalogue of change (109).

Induction of bystander effect, adaptive response and genome instability after xenobiotic exposure can have negative effects on the complexity of foetal development causing imbalance in gene expression (110). This is why future research should investigate adaptive response and its relation to interindividual variability in response to xenobiotics, genome instability, and sensitivity to radiation or chemicals.

Future research should also focus on mechanisms by which hormonal disruptors increase oestrogen, which in turn (111) may interact with radiation and increase cancer risk (112).

Close collaboration of occupational and environmental health specialists with paediatricians, genotoxicologists and oncologists can significantly improve the quality and applicability of available knowledge. Dataset gridding of these four fields could incorporate all aspects of life, from foetal development to parental working environment, and make it possible to evaluate cancer risk for each individual.

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Sažetak**IZLOŽENOST GENOTOKSIČNIM AGENSIMA IZ ŽIVOTNOG OKOLIŠA TIJEKOM PRENATALNOG RAZVOJA I DJETINJSTVA**

Unatoč velikim naporima da se smanji okolišna izloženost u djece se dalje bilježi trend porasta pojavnosti karcinoma i imunskih poremećaja (astma, alergije). Premda su intrauterini razvoj i rano djetinjstvo kritično razdoblje za tumačenje etiologije nastanka karcinoma, transplacentalna i prepubertetna genotoksikologija do danas su slabo istražene. Fetus je transplacentalno izložen brojnim fizikalnim i kemijskim čimbenicima: kontaminantima iz hrane i okoliša, radiokemijski kontaminiranoj vodi, zraku te lijekovima. Ciljna tkiva za djelovanje ksenobiotika mogu biti različita u majke i fetusa zbog različitosti u razvojnoj fiziologiji i distribuciji enzima. Zbog toga u organizmu majke i fetusa mogu nastati različite razine klastogenih i aneugenh metabolita istog ksenobiotika.

Zaštitna uloga odraslih u namjeri da spriječe negativne utjecaje onečišćenog okoliša na djetetovo zdravlje često je ograničena jer su radioizotopi, olovo, PCB, pasivno pušenje, živa, endokrino aktivne tvari, pesticidi i kontaminanti prisutni u svim životnim područjima tijekom razvoja i rasta djeteta. Kako bi se poboljšalo zakonodavstvo vezano uz izloženost djece genotoksičnim i vjerojatno kancerogenim tvarima, tijekom razvoja potrebna je bolja suradnja onkologa, pedijataru, stručnjaka zdravstvene ekologije i genotoksikologa. Na taj način ostvarilo bi se uspješnije iskorištavanje postojećih znanstvenih podataka u cilju smanjenja incidencije karcinoma i mortaliteta.

KLJUČNE RIJEČI: *genotoksikologija djece, ksenobiotik, okolišna izloženost, oštećenje genoma, transplacentalna genotoksikologija*

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