A new database for food safety: EDID (Endocrine disrupting chemicals - Diet Interaction Database)

Francesca Baldi and Alberto Mantovani
Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare, Istituto Superiore di Sanità, Rome, Italy

Summary. Diet is a significant source of exposure to endocrine disrupting chemicals (EDC); health risks cannot be excluded, in particular long-term effects in vulnerable groups such as children. However, food safety assessment must also consider the effects of natural food components modulating the endocrine system. The scientific evidence on the complex interactions between EDC and food components is still limited. The new EDC-Diet Interactions Database (EDID) within the ISS EDC area (www.iss.it/inte/) aims to stimulate further research in the field of food toxicology: a database on international literature's studies, either on experimental systems and on animal population and humans, easy to consult and periodically updated. Examples of studies contained in EDID are provided concerning EDC with iodine, vitamins and phytoestrogens.

Key words: risk assessment, risk-to-benefit-analysis, toxicology, nutrition, endocrine disrupters, contaminants, phytoestrogens.

Riassunto *(Una nuova base di dati sulla sicurezza alimentare: EDID (Endocrine disrupting chemicals - Diet Interaction Database)*. L'alimentazione è un'importante via di esposizione ad interferenti endocrini *(endocrine disrupting chemicals,* EDC), con possibili effetti a lungo termine su gruppi vulnerabili come l'infanzia. Tuttavia la valutazione del rischio in sicurezza alimentare deve anche considerare gli effetti di componenti naturali degli alimenti con potenziali effetti endocrini. Le evidenze scientifiche per caratterizzare le complesse interazioni fra EDC e componenti alimentari sono ancora limitate. Il nuovo database EDC-Diet Interactions Database (EDID) all'interno dell'area tematica "interferenti endocrini" (www.iss.it/inte/) intende contribuire a sviluppare la ricerca nella tossicologia alimentare. EDID comprende articoli internazionali, riguardanti sistemi sperimentali, popolazioni animali e l'essere umano, di facile consultazione ed aggiornata periodicamente. Vengono illustrati esempi di studi presenti in EDID riguardanti le interazioni di EDC con iodio, vitamine e fitoestrogeni.

Parole chiave: valutazione del rischio, analisi rischio-beneficio, tossicologia, alimentazione, interferenti endocrini, contaminanti, fitoestrogeni.

INTRODUCTION

Endocrine disrupting chemicals (EDC) are an heterogeneous group of substances present in the diet and environment able to modify endocrine homeostasis, in particular steroid and thyroid hormones; therefore reproductive health and developing life stages are the most susceptible targets [1]. EDC interact with nuclear receptors or with enzymes responsible of synthesis or transportation of hormones; they include persistent contaminants (*e.g.,* dioxins, polychlorinated biphenyls), compounds used in plant and/or animal production (*e.g.,* dicarboxmides, triazoles) and compounds found in industrial as well as consumer products (*e.g.,* bisphenol A, several phthalates, polybrominated flame retardants) [2]. Endocrine-active chemicals are not confined to xenobiotics. Indeed, many natural bioactive substances may modulate the endocrine system function, from trace elements such as iodine, which are also essential micronutrients [3] to the numerous group of "phytoestrogens". Phytoestrogens are substances present in plants that may interact with estrogen receptors; examples include genistein, an isoflavone found in soy, as well as less well-known compounds present in different grains and fruits, such as daidzein, quercetin, resveratrol, lignans, etc. [4]. EDC arouse concern because of their potential for both long-term, multiple-target effects on vulnerable lifestages as well as for general population exposure associated with the bioaccumulation capability, and/or to widespread diffusion of different compounds [1, 2]. Indeed, EDC exposure in the early lifestages is strongly suspected for the reproductive health deterioration observed in many industrialized Countries

Address for correspondence: Alberto Mantovani, Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: alberto.mantovani@iss.it.

[5]. Consequently EDC are one of the chemical contamination problems that drew great attention by the scientific community and public opinion. This is reflected, at European level, by the novel developments of testing strategies, especially in the field of reproductive and developmental toxicology [6], as well as by the many research projects on EDC [7]. A specific task force on testing and assessment of EDC has also been set up within the chemical programme of the Organisation for Economic Cooperation and Development (OECD) [8]. Due to the high and up-to-date relevance of EDC research to the fields of food and environmental safety as well as of preventive medicine, the Istituto Superiore di Sanità (ISS) has launched since 2002 a dedicated website, that is updated weekly, with a full English version and a special interest to collect information on ongoing Italian research as well as on emerging topics (http://www.iss.it/inte).

ENDOCRINE DISRUPTING CHEMICALS, DIET AND NUTRIENTS

Diet is a particularly significant source of exposure to EDC for the general population. The suspiciousness of EDC assumption via diet is a source of concerns and "alarms" more or less justified for consumers' health. One major issue is the "cocktail" effect. Residue monitoring data in Europe indicate that regulatory limits are exceeded in a minority of cases, at least concerning compounds for which such limits do exist, *e.g.* pesticides [9]; however, one cannot rule out altogether additivity of different EDC present in whole diet at low-level, but hitting the same targets, *e.g.* nuclear receptors [10]. Furthermore, it is not just the daily dose that matters. Many EDC can bioaccumulate, especially in body lipids, forming a "body burden" of contaminants of different origin that can include dioxins, banned compounds that still persist in the environment (polychlorinated biphenyls, chlorinated pesticides and their metabolites), and brominated flame retardants [11]. Other compounds, even though less persistent, may nonetheless concentrate in food chains and/or body tissues, thus adding to the overall EDC internal burden; examples are phthalates [12] and organotins [13]. Thus, the potential risk is less related to the contamination of a single food commodity than to the long term intake through the overall diet, whose impacts on the total EDC body burden as well as on chronic health effects are incompletely known. Another issue to be considered in risk assessment is different vulnerabilities within the general population: concerning EDC, those best recognized are related to age and gender. For instance, children can be considered as a group at higher risk, due to higher relative consumption of food and water as compared to adults as well as to the ongoing functional development of reproductive, nervous and immune systems [14].

However, other factors that can modulate vulnerability deserve adequate consideration. The modern conception of food toxicology cannot consider diet just as an exposure source of external harmful substances. Contaminants such as EDC may interact with the same metabolic pathways as natural food components such as polyunsaturated fatty acids, trace elements, vitamins and other bioactive substances (*e.g.* polyphenols) that cannot be considered nutrients as there is no recognized deficiency. Dietary habits are related to socioeconomic status, cultural and religious factors, individual choices (*e.g.* vegetarianism/veganism); in their turn dietary habits may have a most important impact on the intake of either nutrients and contaminants. For instance, the changing dietary habits in Italy lead to a lower intake of specific vitamins (folic acid, B6 and E) as compared to that expected in the traditional "Mediterranean diet" [15]; also, greater exposure to persistent EDC is associated with the high consumption of fatty foods of animal origin [16]. Thus, for specific food commodities a balanced evaluation is needed about contaminant-associated risks and nutritional benefits. The most up-to-date example is represented by salmonids and other seafood, both wild and farmed, that are a useful source of nutrients such as polyunsaturated fatty acids as well as a major source of exposure to EDC and other contaminants able to bioaccumulate such as methylmercury; therefore, evidence might justify recommendations to increase as well as to reduce fish consumption, quite an uneasy situation for risk managers and public health policy makers [17]. Indeed, as response to several pollution "alarms" in northern Countries, the European Food Safety Authority (EFSA) issued in 2005 its first risk-to-benefit opinion about consumption of wild and farmed fish [18]. The EFSA document concluded that decreasing fish consumption (and its nutritional benefits) is not necessary in Europe; in the meanwhile, recommendations included to continue the monitoring of contaminants in edible fish as well as the support to development of novel aquaculture feeds, less liable to contamination. Most important, effects of contaminants and of natural food components are not running in parallel; in most instances, they interact on the same pathways and targets. It can be inferred that a well-balanced diet, with a good intake of antioxidants, may be partly protective towards the effects of toxic exposures. The interactions may not be always so straightforward, however, as several nutrients and bioactive components can exert adverse effects. An example are phytoestrogens, that can offer a protective effect against some hormone-dependent cancers, as well as postmenopausal osteoporosis, but may also interfere with receptor-mediated signal transduction (*e.g.* by inhibiting protein kinase) and DNA replication, which might also lead to promoting the proliferation of some cancer cells [19]. One cannot even exclude that high phytoestrogen dosage may have additive effects associated with contaminants that target the same receptors. Up to date, scientific data available on interactions between xe-

nobiotics and "natural" substances in food are still meagre, in spite of the relevance that such topic may have in the food safety and prevention fields.

EDID: ENDOCRINE DISRUPTING CHEMICALS - DIET INTERACTION DATABASE

The new EDC - Diet Interactions Database (EDID) within the ISS EDC website (www.iss.it/inte/edid/cont.php?id=110&lang=2&tipo=17), started in 2006, is the first database dedicated to interactions between EDC and nutrients; currently it contains more than 300 items. For setting and updating the database the terms "EDC" and "diet" needed definition. EDC is taken in its broad sense, as in the 1996 Weighbridge definition adopted in Europe as a chemical that can induce adverse effects on the health of an organism or of its progeny through endocrine mechanisms (see the ISS EDC website homepage www.iss.it/inte/?lang=2; whereas "diet" includes all nutrients, either macro- (protein, glucose, etc.) or micronutrients (vitamins, trace elements), as well as bioactive compounds that can be naturally present in foods, besides any man-made addition. EDID aims to be a contribution for stimulating further research in the field of xenobiotics-nutrients interactions. EDID is conceived primarily as a database on studies published on international peer-reviewed literature, either on experimental systems *in vitro*/*in vivo*, animal populations (both wild and farmed) and humans. However, some relevant reports available on the web and issued by national or international Agencies are also included, such as the already quoted EFSA opinion on fish consumption [18]. EDID aims at being a user-friendly database; it utilizes DSpace which is one of the first open source software platforms to store, manage and distribute its collections in digital format (http://dspace.iss.it/dspace/). Different search criteria may be set, namely: a) words present in the article's title; b) keywords not present in the article's title, including s, nutrients, animal species, targets (tissues, organs, enzymes, receptors etc.); usually four such keywords are selected for each paper; c) the author's name (the first three if more than three authors are present); d) the article's release year. Due to current copyright regulations, most documents present in EDID are available only as abstracts: however, the corresponding author's address and full journal reference enables to easily recover the full paper, when needed.

EDID is periodically updated. The current lack of standard keywords or search tools for papers on toxicant-nutrient interactions makes it necessary to perform an expert-based, case-by-case "fishing" search. For instance, the keywords "endocrine disrupter", "endocrine disruptor" or "endocrine disrupting chemical" do not catch many papers on dioxins, polychlorinated biphenyls or phytoestrogens, although such keywords do perform more effectively with chemicals like phthalates, bisphenol A or pesticides; the reason might be that phytoestrogens are not typical EDC, while investigations on dioxins and PCB took course years before the widespread diffusion of the EDC concept. A simple, general keyword like "endocrine" matched with nutrient-relevant keywords ("trace element", "vitamin", nutrient" or more specific ones) can be more effective, although it obviously lacks specificity and requires careful selection of the really EDID-relevant papers. Currently, updating has to be performed through a selection of matched keywords (*e.g.,* "PCB AND vitamin", "Pesticide" AND "phytoestrogen", etc.), extracting the relevant papers and the "fishing" collection of related papers. Although such approach has been satisfactorily effective in the first launching phase of EDID, that will end 24 months after start, the elaboration of a more systematic approach to literature search will be implemented in the following phases.

EXAMPLES OF EDID TOPICS

The following paragraphs deal with some of the main topics that can be found in the EDID database.

Iodine, thyroid function and EDC

Iodine is the main determinant of thyroid development and function; some food commodities of animal origin, namely seafood and milk, are the main dietary sources, nevertheless subclinical iodine deficiency in humans and farm animals is still a common problem in many areas, including Europe [3]. Thyroid is also an emerging target for EDC; it may be reasonable to hypothesize that a low iodine status would enhance the susceptibility to thyrostatic xenobiotics. Yet, it is unfortunate that only a limited number of papers till now deal with the relationship between iodine status and EDC effects. Somewhat unexpectedly phthalates, widespread plasticizers that rise concern mainly because of their antiandrogenic effects, can modulate basal iodide uptake mediated by the sodium/iodide symporter in thyroid follicular cells *in vitro* [20]. Interestingly, the effect was not a general effect of phthalates and was independent from cytotoxicity; dibutyl phthalate was the most cytotoxic out of the six compounds tested but it did not modulate iodide uptake. Phytoestrogens may also deserve more investigation as a study show that many of them may interfere with iodination of thyroid hormones: some (*e.g.,* naringenin, and quercetin, which contain a resorcinol moiety) are direct and potent inhibitors of thyroid peroxidase, whereas others (myricetin, naringin) showed noncompetitive inhibition of tyrosine iodination with respect to iodine ion and biochanin A acted as an alternate substrate for iodination [21]. Indeed, a Czech biomonitoring study in children without over hypothyroidism showed a modest but significant adverse relationship of serum concentrations of genistein, with markers of thyroid function, while no significant association was found for another isoflavone, daidzein. However, since even small differences in

phytoestrogen intake were related to thyroid function, this might have an impact in children populations where iodine intake is insufficient [22].

Oxidative stress, antioxidant vitamins and EDC

Activation of nuclear receptors such as the estrogen receptor alpha is closely related to redox status and oxidative stress pathways [23]. Indeed, one of the major clusters within EDID includes papers showing that antioxidants such as vitamins C and E exert a protective role towards the effects of EDC such as phthalates and PCB. Two examples are given. Di-(2-ethylhexyl) phthalate (DEHP) may be the most potent EDC among phthalates and it is surely the most thoroughly investigated; the compound can interfere with steroid synthesis pathways by interacting with the Pregnane-X nuclear receptor [24]. DEHP can severely disrupt spermatogenesis in adult rodents; however such effects are reversible upon discontinuation of the treatment. Supplementation with vitamins C and E can protect the seminiferous epithelium from DEHP effects; moreover, the supplementation with both vitamins after the treatment significantly accelerated regeneration of the testis epithelium [25]. Non-dioxin like PCB can alter endocrine homeostasis through several, mainly non-receptor mediated, mechanisms. The PCB mixture Aroclor 1254 was tested on cultured chicken embryo hepatocytes with and without vitamin E, vitamin C and vitamin A, alone and in combination. Aroclor 1254 caused irreversible damage to cell membrane integrity and increased cellular lipid peroxidation, whilst reducing glutathione levels and superoxide dismutase activities. The vitamins, either alone and in combination (A+E and C+E, apparently not A+C) significantly attenuated the toxic effects; the findings in this system also suggest that lipid peroxidation may be a key event in PCB toxicity [26]. Still most studies on EDC and antioxidant vitamins show a protective effect, but only few attempts to elucidate mechanisms. Also, it would be interesting to have more comprehensive data on the combined impact on EDC effects of dietary antioxidants, in the proportion and amount provided by the different dietary habits [27].

EDC and vitamin A

Retinoic acid is the internal form of vitamin A interacting with the nuclear receptors RAR and RXR; all-trans-retinoic acid and 9-cis-retinoic acid are the natural ligands for RAR and RXR, respectively. In its turn, retinoic acid pathways cross-talk with that of the aryl hydrocarbon receptor (AhR), the direct cell target for dioxins and dioxin-like compounds [28]. Thus, it is relevant to investigate the specific interactions between EDC and vitamin A metabolism. However, up to now only a few have been clearly shown to interfere with this pathway. The most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent inducer of cytochrome P450 (CYP) 1A1, that in its turn may enhance the toxic effects of TCDD. Several studies indicated that concurrent supplementation of vitamin A could inhibit CYP1A1 activity (measured as ethoxyresorufin-O-deethylase [EROD] activity). In particular, in mice orally treated with TCDD the concurrent administration of vitamin A reduces liver damage and the specific markers of TCDD action, namely the augmented EROD activities, CYP1A1 expression, and AhR mRNA expression [29]. These findings suggest a relationship between vitamin A depletion and TCDD effects at molecular levels, at least in liver tissue; however, the role of retinoic acid as a target within the wide range of adverse dioxin-induced effects still needs clarification. Organotins do not bind to steroid nuclear receptors, but may alter steroid biosynthesis [13]. Tributyltin chloride and triphenyltin hydroxide, bind specifically to the RXR in human choriocarcinoma cells; binding elicit transcriptional activation of endocrine signalling, *e.g.* aromatase, but not the retinoic acid pathways [30]. Thus, more research on the interactions of EDC acting through different mechanisms with retinoic acid pathways could be warranted.

Phytoestrogens and xenobiotics

Due to their pleomorphic biological effects, including some potentially adverse ones, phytoestrogens could be considered a sort of "natural EDC" [4, 19]. Most attention has been concentrated on the soy phytoestrogen genistein, possibly the most potent phytoestrogen; however, the overall dietary intake of phytoestrogens may be significant in Europe, even though lower than in Eastern Asia [31]. Therefore the interactions between phytoestrogens and EDC could represent a major topic of interest, due to combined presence in diet and possible sharing of biological targets. Yet, periodical EDID updating has led to include less than 20 papers on such issue till now. As phytoestrogens may have beneficial effects on human health, they have been also hypothesized to compete with estrogenic EDC, thus exerting a protective action. Indeed, flavonoids (daidzein, genistein, quercetin, and luteolin) can suppress the induction of the proliferation-stimulating activity of estrogen-like acting EDC (4-nonylphenol, bisphenol A, and the PCB 4-dihydroxybiphenyl) in MCF-7 cells [32]. The effect of mixtures containing 17beta-estradiol, 17alpha-ethinylestradiol, genistein, and estrogenic EDC (the plastic additive bisphenol A, and the anionic detergent by-products 4-nonylphenol, and 4-tert-octylphenol.) was less than additive when assessed through the proliferation of estrogen-dependent MCF-7 human breast cancer cells; the partly anatagonistic interaction was observed in particular between the other compounds, including genistein, and 4-nonylphenol and 4-tert-octylphenol [33]. In some cases no interaction can be observed: the combined treatment with genistein did not modulate the *in vitro* effects on human astroglial cells

of two persistent EDC, the polybrominated flame retardant PBDE-99 and the PCB mixture Aroclor 1254 [34]. The available findings *in vivo* provide a more complex picture. Genistein and the estrogenic chlorinated insecticide methoxychlor were tested on rats alone or in combination, to assess possible effects on immune function, a potential critical target for EDC that is still somewhat underscored by the available literature. A possible additive effect was seen in the parental generation on the reduction of relative spleen and thymus weight as well as, to a lesser extent, of weight gain. In the F1 generation the combined effect of genistein (at dose levels lower than those effective in the parental generation) and methoxychlor had a significant detrimental impact on the total number of thymocytes as well as on the different CD4-CD8 subclasses [35]. New insights are provided by a recent paper using estrogen reporter (ERE-tK-Luciferase) male mice. Genistein can partially accumulate in body fat depots where it may persist for weeks at functionally active levels; following fasting genistein is released from adipose tissue in the circulation and can modulate the actions of either estradiol and persistent EDC in liver and testis with tissue-specific features. In particular genistein reversed the antiestrogenic action of beta-benzene-hexachloride in the tesis and of o,p'-DDT in the liver, whereas it had an additive effect with the ER agonist p,p'-DDT in the liver [36]. Thus, the available data can only indicate that interactions between phytoestrogens and EDC can be important but also that they cannot simply explained in terms of additivity or antagonism; as a consequence the dietary intake of phytoestrogens cannot be considered *per se* a protective factor towards exposures to environmental EDC. More research should be warranted on phytoestrogens other than genistein as well as biomonitoring studies in humans on both ohytostrogens and contaminants using appropriate biomarkers.

CONCLUSIONS

EDID represents a novel instrument for research in food toxicology, that will be implemented and increased as the EDID issues will receive increasing attention from research and risk assessment. The shortcomings of EDID are these of the available literature. As no systematic projects or actions do exist yet on the interface between EDC and dietary components, it is unavoidable that the presence of a given topic is related to the interest (and success) of a given research group. Examples, as mentioned above, are the paucity of studies on iodine uptake modulation by EDC and the comparative abundance of papers investigating the putative protective role of the antioxidant vitamins C and E. Also, a large proportion of papers present in EDID deal with "old" contaminants such as dioxins, PCB and to a lesser extent heavy metals; other, more recent EDC such as phthalates, bisphenol A, nonylphenol, polyborominated flame retardants or organotins are much less represented, even though their current exposure levels do create concerns for food and environmental safety [11-13]. It is also somewhat surprising that a number of papers on toxicant (including EDC)-nutrient interactions were issued some 10-20 years ago, *i.e.*, before the start or the full development of interest and research on EDC. Such papers, even though valuable, obviously cannot provide the in-depth information that could be supported by the novel research tools of molecular toxicology [27-33], and that is required by the conceptual framework of EDC risk assessment. Nevertheless, apparently the topic did not draw too much attention from risk assessment or research-funding agencies; this situation may rapidly change, due to food safety becoming a cutting-edge topic for both research and public health as well as the new developments of risk-to-benefit analysis. Indeed, as other topics become "fashionable", a new output of papers is expected, *e.g.* on risk-to-benefit analysis of contaminants and nutrients in seafood [17, 18]. Other shortcomings of the database reflect more wider knowledge gaps. For instance, the soy isoflavone genistein is the only phytoestrogen that is thoroughly investigated, even though other compounds, *e.g.* lignans, may have a higher intake from European populations [31]. Most important, there are still a limited number of human epidemiological studies that concurrently investigated markers of toxicant exposures and of nutrient intake. Examples include: the assessment of long-term perchlorate exposure thorough drinking water, thyroid markers and iodine in urine and breast milk that has been carried out in Northern Chile and found no significant impact [37]; the intervention study with folic acid, carried out on adults from an arsenic-polluted area of Bangladesh, indicating that folic acid supplementation enhances arsenic methylation, hence metabolism and excretion, in subjects with low plasma folate [38]. Therefore, a full assessment of EDID content, development accessibility and usability will be performed after the first 24 month-phase of implementation.

Yet, notwithstanding the unavoidable problems of a starting phase, increasing evidence supports the hypothesis originating EDID, *i.e.*, that diet is a substantial risk modulator of EDC-related health risks and that an evidence-based assessment of the complex EDC-diet interactions is required [31, 33, 34]. Thus, in perspective EDID may provide important information and tools also to preventive medicine, namely i) nutritional markers that are relevant to the susceptibility to specific EDC or EDC groups [37] and ii) dietary interventions or supplementation that may be performed with population groups exposed to EDC that interfere with metabolism of specific nutrients [38].

Finally, as pointed out by the EFSA [39], new approaches are needed in food safety, encompassing also a comprehensive of the health impact – either

detrimental and/or beneficial – of a given food commodity. When required, decision makers should be provided with a balanced assessment of toxicological and nutritional risks and benefits; thus the development of a knowledge basis on toxicant-nutrient interactions is most critical for a science-supported risk assessment in food safety.

Acknowledgements

EDID has been elaborated within the frame of the ISS special Project "Interferenti Endocrini" (resp. A. Mantovani) as well as of the 6th FP Network of Excellence CASCADE (www.cascadenet.org).

Submitted on invitation.
Accepted on 16 December 2007.

References

1. Mantovani A. Risk assessment of endocrine disrupters. The role of toxicological studies. *Ann N Y Acad Sci* 2006;1076:239-52.
2. Mantovani A. Hazard identification and risk assessment of endocrine disrupting chemicals with regard to developmental effects. *Toxicology* 2002;181-182:367-70.
3. European Food Safety Authority. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the request from the Commission on the use of iodine in feedigstuffs. *EFSA J* 2005;168:1-42.
4. Saarinen NM, Bingham C, Lorenzetti S, Mortensen AA, Mäkela S, Penttinen P, Sorensen IK, Valsta LM, Virgili F, Vollmer G, Wärri A, Zierau O. Tools to evaluate estrogenic potency of dietary phytoestrogens: a consensus paper from the EU thematic network "Phytohealth" (QLKI-2002-2453). *Genes Nutri* 2006;1:143-58.
5. Sharpe RM. The "oestrogen hypothesis" where do we stand now? *Int J Androl* 2003;26:2-15.
6. Bremer S, Cortvrindt R, Daston G, Eletti B, Mantovani A, Maranghi F, Pelkonen O, Ruhdel I, Spielmann H. *Reprod Develop Tox Altern Lab Anim* 2005;1:183-209.
7. European Commission. Directorate-General of Research. *Endocrine disrupters research in the European Union*. Available from: http://ec.europa.eu/research/endocrine/index_en.html.
8. Gelbke HP, Kayser M, Poole A. OECD test strategies and methods for endocrine disruptors. *Toxicology* 2004;205:17-25.
9. Rescia M, Mantovani A. Pesticides as endocrine disrupters. Identification of hazards for female reproductive function. In: Nicolopoulou-Stamati P, Hens L, Howard CV (Ed.). *Reproductive health and the environment*. Dordrecht (NL): Springer; 2007. (Series: Environmental Science and Technology Library, Vol. 22) p. 161-86.
10. Willingham E. Endocrine-disrupting compounds and mixtures: unexpected dose-response. *Arch Environ Contam Toxicol* 2004;46:265-9.
11. Fromme H, Albrecht M, Angerer J, Drexler H, Gruber L, Schlummer M, Parlar H, Korner W, Wanner A, Heitmann D, Roscher E, Bolte G. Integrated Exposure Assessment Survey (INES) exposure to persistent and bioaccumulative chemicals in Bavaria, Germany. *Int J Hyg Environ Health* 2007;210:345-9.
12. Koch HM, Preuss R, Angerer J. Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure – an update and latest results. *Int J Androl* 2006;29:155-65.
13. European Food Safety Authority. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission to assess the health risks to consumers associated with exposure to organotins in foodstuffs. *EFSA J* 2004;102:1-119.
14. Landrigan PJ, Kimmel CA, Correa A, Eskenazi B. Children's health and the environment: public health issues and challenges for risk assessment. *Environ Health Perspect* 2004;112:257-65.
15. Sofi F, Vecchio S, Giuliani G, Martinelli F, Marcucci R, Gori AM, Fedi S, Casini A, Surrenti C, Abbate R, Gensini GF. Dietary habits, lifestyle and cardiovascular risk factors in a clinically healthy Italian population: the "Florence" diet is not Mediterranean. *Eur J Clin Nutr* 2005;59:584-91.
16. Fattore E, Fanelli R, Turrini A, Di Domenico A. Current dietary exposure to polychlorodibenzo-p-dioxins, polychlorodibenzofurans, and dioxin-like polychlorobiphenyls in Italy. *Mol Nutr Food Res* 2006;50:915-21.
17. Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA* 2006;296:1885-99.
18. European Food Safety Authority. Opinion of the Scientific Panel on Contaminants in the food chain on a request from the European Parliament related to the safety assessment of wild and farmed fish. *EFSA J* 2005;236:1-118.
19. Martin JH, Crotty S, Nelson PN. Phytoestrogens: perpetrators or protectors? *Future Oncol* 2007;3:307-18.
20. Wenzel A, Franz C, Breous E, Loos U. Modulation of iodide uptake by dialkyl phthalate plasticisers in FRTL-5 rat thyroid follicular cells. *Mol Cell Endocrinol* 2005;244:63-71.
21. Divi RL, Doerge DR. Inhibition of thyroid peroxidase by dietary flavonoids. *Chem Res Toxicol* 1996;9:16-23.
22. Milerová J, Cerovská J, Zamrazil V, Bílek R, Lapcík O, Hampl R. Actual levels of soy phytoestrogens in children correlate with thyroid laboratory parameters. *Clin Chem Lab Med* 2006;44:171-4.
23. Roy D, Cai Q, Felty Q, Narayan S. Estrogen-induced generation of reactive oxygen and nitrogen species, gene damage, and estrogen-dependent cancers. *J Toxicol Environ Health B Crit Rev* 2007;10:235-57.
24. Hurst CH, Waxman DJ. Environmental phthalate monoesters activate pregnane X receptor-mediated transcription. *Toxicol Appl Pharmacol* 2004;199:266-74.
25. Ablake M, Itoh M, Terayama H, Hayashi S, Shoji S, Naito M, Takahashi K, Suna S, Jitsunari F. Di-(2-ethylhexyl) phthalate induces severe aspermatogenesis in mice, however, subsequent antioxidant vitamins supplementation accelerates regeneration of the seminiferous epithelium. *Int J Androl* 2004;27:274-81.
26. Zhou C, Zhang C. Protective effects of antioxidant vitamins on Aroclor 1254-induced toxicity in cultured chicken embryo hepatocytes. *Toxicol In Vitro* 2005;19:665-73.
27. Ruhl R. Effects of dietary retinoids and carotenoids on immune development. *Proc Nutr Soc* 2007;66:458-69.
28. Murphy KA, Quadro L, White LA. The intersection between the aryl hydrocarbon receptor (AhR) and retinoic acid-signaling pathways. *Vitam Horm* 2007;75:33-67.
29. Yang YM, Huang DY, Liu GF, Zhong JC, Du K, Li YF, Song XH. Inhibitory effects of vitamin A on TCDD-induced cytochrome P-450 1A1 enzyme activity and expression. *Toxicol Sci* 2005;85:727-34.
30. Nakanishi T, Nishikawa J, Hiromori Y, Yokoyama H, Koyanagi M, Takasuga S, Ishizaki J, Watanabe M, Isa S, Utoguchi N, Itoh N, Kohno Y, Nishihara T, Tanaka K. Trialkyltin compounds bind retinoid X receptor to alter human placental endocrine functions. *Mol Endocrinol* 2005;19:2502-16.

31. Peeters PH, Slimani N, van der Schouw YT, Grace PB, Navarro C, Tjonneland A, Olsen A, Clavel-Chapelon F, Touillaud M, Boutron-Ruault MC, Jenab M, Kaaks R, Linseisen J, Trichopoulou A, Trichopoulos D, Dilis V, Boeing H, Weikert C, Overvad K, Pala V, Palli D, Panico S, Tumino R, Vineis P, Bueno-de-Mesquita HB, van Gils CH, Skeie G, Jakszyn P, Hallmans G, Berglund G, Key TJ, Travis R, Riboli E, Bingham SA. Variations in plasma phytoestrogen concentrations in European adults. *J Nutr* 2007;137:1294-300.

32. Han DH, Denison MS, Tachibana H, Yamada K. Relationship between estrogen receptor-binding and estrogenic activities of environmental estrogens and suppression by flavonoids. *Biosci Biotechnol Biochem* 2002;66:1479-87.

33. Rajapakse N, Silva E, Scholze M, Kortenkamp A. Deviation from additivity with estrogenic mixtures containing 4-nonylphenol and 4-tert-octylphenol detected in the E-SCREEN assay. *Environ Sci Technol* 2004;38:6343-52.

34. Madia F, Giordano G, Fattori V, Vitalone A, Branchi I, Capone F, Costa LG. Differential *in vitro* neurotoxicity of the flame retardant PBDE-99 and of the PCB Aroclor 1254 in human astrocytoma cells. *Toxicol Lett* 2004;154:11-21.

35. Guo TL, Zhang XL, Bartolucci E, McCay JA, White KL Jr, You L. Genistein and methoxychlor modulate the activity of natural killer cells and the expression of phenotypic markers by thymocytes and splenocytes in F0 and F1 generations of Sprague-Dawley rats. *Toxicology* 2002;172:205-15.

36. Penza M, Montani C, Romani A, Vignolini P, Ciana P, Maggi A, Pampaloni B, Caimi L, Di Lorenzo D. Genistein accumulates in body depots and is mobilized during fasting, reaching estrogenic levels in serum that counter the hormonal actions of estradiol andorganochlorines. *Toxicol Sci* 2007;97:299-307.

37. Téllez Téllez R, Michaud Chacón P, Reyes Abarca C, Blount BC, Van Landingham CB, Crump KS, Gibbs JP. Long-term environmental exposure to perchlorate through drinking water and thyroid function during pregnancy and the neonatal period. *Thyroid* 2005;15:963-75.

38. Gamble MV, Liu X, Ahsan H, Pilsner JR, Ilievski V, Slavkovich V, Parvez F, Chen Y, Levy D, Factor-Litvak P, Graziano JH. Folate and arsenic metabolism: a double-blind, placebo-controlled folic acid-supplementation trial in Bangladesh. *Am J Clin Nutr* 2006;84:1093-101.

39. European Food Safety Authority. *EFSA scientific colloquium summary report 6. Risk-benefit analysis of foods: Methods and approaches.* EFSA; 13-14 July 2006. Available from: http://www.efsa.europa.eu/EFSA/Scientific_Document/comm_colloque_6_en.pdf; last visited 21/11/2007.