Environmental Research 151 (2016) 91–100

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Novel application of statistical methods for analysis of multiple toxicants identifies DDT as a risk factor for early child behavioral problems

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ARTICLE INFO

Article history:
Received 26 February 2016
Received in revised form
7 July 2016
Accepted 11 July 2016

Keywords:
Nervous system
Child development
Dichlorodiphenyl dichloroethylene
Dichlorodiphenyldichloroethane
Chlordane
Hexachlorobenzene
Polychlorinated biphenyls
Polybrominated diphenyl ethers
Complex mixtures

ABSTRACT

Background: The aim of this study was to assess the association between postnatal exposure to multiple persistent organic pollutants (POPs) measured in breast milk samples and early behavioral problems using statistical methods to deal with correlated exposure data.

Methods: We used data from the Norwegian HUMIS study. We measured concentrations of 24 different POPs in human milk from 612 mothers (median collection time: 32 days after delivery), including 13 polychlorinated biphenyls (PCB) congeners, 6 polybrominated diphenyl ethers (PBDE) congeners and five organochlorine compounds. We assessed child behavioral problems at 12 and 24 months using the infant toddler symptom checklist (ITSC). Higher score in ITSC corresponds to more behavioral problems. First we performed principal component analysis (PCA). Then two variable selection methods, elastic net (ENET) and Bayesian model averaging (BMA), were applied to select any toxicants associated with behavioral problems. Finally, the effect size of the selected toxicants was estimated using multivariate linear regression analyses.

Results: *p,p′*-DDT was associated with behavioral problems at 12 months in all the applied models. Specifically, the principal component composed of organochlorine pesticides was significantly associated with behavioral problems and both ENET and BMA identified *p,p′*-DDT as associated with behavioral problems. Using a multiple linear regression model an interquartile increase in *p,p′*-DDT was associated with a 0.62 unit increase in ITSC score (95% CI 0.45, 0.79) at 12 months, corresponding to more behavioral problems. The association was modified by maternal education: the effect of *p,p′*-DDT was strongest in women with lower education (β=0.59; 95%CI: 0.38, 0.81) compared to the mother with higher education (β=0.14; 95%CI: −0.05, 0.34) (*p*-value for interaction=0.089). At 24 months, neither selection method consistently identified any toxicant associated with behavioral problems.

Conclusion: Within a mixture of 24 toxicants measured in breast milk, *p,p′*-DDT was the single toxicant associated with behavioral problems at 12 months using different methods for handling numerous correlated exposures.

Abbreviations: POPs, persistent organic pollutants; PCBs, polychlorinated biphenyls; p,p′-DDT, 1,1,1-trichloro-2,2-bis (4chlorophenyl) ethane; p,p′DDE, 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene; HCB, Hexachlorobenzene; ß-HCH, ß-hexachlorocyclohexane; PBDEs, polybrominateddiphenyl et; PCA, principal component analysis; ENET, elastic net; BMA, Bayesian model averaging; HUMIS, Norwegian Human Milk Study; NIPH, Norwegian Institute of Public Health; GC–MS, liquid-liquid extraction and gas chromatography – mass spectrometry; LOD/LOQ, limit of detection/quantification; ITSC, Infant/Toddler Symptoms Checklist; MBR, Medical Birth Registry of Norway; BMI, body mass index; SCL-5, Hopkins Symptom Checklist; IQR, interquartile range; DAG, directed acyclic graph; PCs, Principal Components; CI, Confidence Interval; PIP, posterior inclusion probability; MSE, mean-squared error

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http://dx.doi.org/10.1016/j.envres.2016.07.014
0013-9351/

1. Introduction

Persistent organic pollutants (POPs) are environmental chemicals with well-documented toxic potency. They are resistant to degradation in nature, accumulate in wildlife and humans and biomagnify through food chains. POPs encompass a wide variety of chemicals including organochlorines [polychlorinated biphenyls (PCBs), 1,1,1-trichloro-2,2-bis (4chlorophenyl) ethane (p,p′-DDT), and 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene (p,p′DDE), hexachlorobenzene (HCB), and ß-hexachlorocyclohexane (ß-HCH)], brominated compounds [polybrominateddiphenyl ethers (PBDEs)]] and many other chemicals. Fetuses and newborns are exposed to POPs through placental transfer and breastfeeding (Bergonzi et al., 2009; Dingemans et al., 2011; Ribas-Fito et al., 2001). Many POPs have been banned across much of the world (Stockholm Convention on Persistent Organic Pollutants (POPs, 2009), and thus, concentrations of PCBs, *p,p′*-DDE and HCB in humans have decreased over time (Nickerson, 2006). However, most of these compounds are still detectable in the blood of current generations due to their persistence in the environment and their long half-lives in humans (Jönsson et al., 2005; Toms et al., 2009).

PCBs, PBDEs, *p,p′*-DDE, HCB are considered developmental neurotoxicants (Grandjean and Landrigan, 2014), mainly based on studies looking at the association between *in utero* exposure to single pollutants and neuropsychological development. However, children are exposed to multiple toxicants, which coexist in exposure sources and are, therefore, often highly correlated. To date, few studies have used a multipollutant approach to study the association between toxicants and health (Agay-Shay et al., 2015; Braun et al., 2014; Grandjean et al., 2012; Lee et al., 2007; Lenters et al., 2015b; Patel et al., 2010). In general, there is a lack of knowledge on the potential neurotoxicity of mixtures, and in particular that resulting from exposure through breastfeeding.

We aimed to assess the association between postnatal exposure to 24 different POPs measured in milk samples and early behavioral problems at 12 and at 24 months of age, using the longitudinal HUMIS birth cohort. We employed principal component analysis (PCA), elastic net (ENET) and Bayesian model averaging (BMA) in order to identify toxicants potentially associated with behavioral problems in early life. Then, we used traditional multivariate regression methods to estimate the association between selected toxicant(s) and early behavioral problems.

2. Methods

2.1. Study population

The “Norwegian Human Milk Study” (HUMIS) is a multi-center birth cohort of mother-child pairs recruited between 2002 and 2009. Within approximately two weeks of giving birth, mothers were recruited by public health nurses during a routine home visit to all new mothers in Norway, except in Østfold county where mothers were recruited at the maternity ward, two consecutive term births for every preterm. Participants were asked to collect a 25 ml breast milk sample each morning for eight consecutive days, preferably sampled between 2 weeks and two months in line with the WHO recommendation, but they were informed that milk sampled otherwise was also accepted, but changes in sampling protocol were noted. The milk was stored in a 250 ml container kept in the freezer. Date and time of collection were recorded for each sample, as well as whether a breast pump had been used. When the container had been filled, participants mailed it by regular mail, except in the county of Østfold where the milk samples were collected by study personnel and kept frozen during transport to the Norwegian Institute of Public Health (NIPH). Further details have been published elsewhere (Eggesbø et al., 2011).

Informed consent was obtained prior to the study and the study was approved by the Norwegian Data Inspectorate and Regional Ethics Committee for Medical Research. Overall, 36% of the invited women declined to participate in the study.

So far, among the 2.606 participants in the HUMIS study, 612 women have had their milk samples analyzed for the complete list of POPs (due to financial constraints not everyone could be analyzed at once): 498 were randomly selected; 54 were oversampled based on preterm status and 60 were oversampled due to rapid growth of their infant (Fig. 1). Among these 612 subjects 52 and 78 had not sent in the 12 and 24 month questionnaires, respectively. In addition, there were some with missing values on the specific questions needed for the neuropsychological assessments (12 and 6, for 12 and 24 month questionnaires, respectively) (Fig. 1). Therefore, the final subsets used in this study were 548 and 528, for 12 and 24 months respectively.

2.2. Exposure measurement

The POPs concentrations were determined in breast milk. The median age of child at start of sampling was 32 days after delivery (min 2 days, max 171 days). The chemical measurements were performed in two laboratories. Briefly, the concentrations of six PBDE congeners (PBDE 28, 47, 99, 100, 153 and 154) were determined in 612 samples at the Department of Exposure and Risk Assessment, NIPH using liquid-liquid extraction and gas chromatography (GC) – mass spectrometry (MS) with negative chemical ionization as described in detail elsewhere (Thomsen et al., 2010, 2007).

Concentrations of HCB, β-HCH, *p,p′*-DDE, *p,p′*-DDT, oxychlordane (oxyCD), seven non-dioxin-like polychlorinated biphenyls (ndl-PCBs) (IUPAC nos.: 74, 99, 138, 153, 170, 18, and 194), and six dioxin-like mono-ortho PCBs (mo-PCBs) (IUPAC nos.: 105, 114, 118, 156, 157 and 189) were measured at the Department of Exposure and Risk Assessment, NIPH (Thomsen et al., 2007) in 532 samples using liquid-liquid extraction and GC–MS with negative chemical ionization, and at the University of Life Science-NMBU in a further 80 samples using liquid/liquid extraction, gravimetrical lipid determination and clean-up with sulfuric acid (Eggesbø et al., 2011, 2009; Polder et al., 2009, 2008). All the methods used are described in more detail in supplemental material (Supplemental Methods S1). We replaced POP concentrations below the limit of detection/quantification (LOD/LOQ) with a randomly generated number between 0 and the analysis-specific LOQ.

2.3. Behavioral assessment

We assessed the behavioral problems at 12 and 24 months using a subset of items from the Infant/Toddler Symptoms Checklist (ITSC): long version (De Gangi and Poisson, 2000), which mothers completed. The ITSC is a questionnaire that assesses self-regulation and aspects of temperament, and identifies any regulatory problems that may be arising, such as fussiness, going quickly from a whimper to a loud cry, and sleeping and eating difficulties in children aged 7–30 months. For the present study, we included questions on self-regulation, attention, sleep, eating or feeding, dressing-bathing-touch, and listening-language-sound subscales. For each item, the child is rated as “never” or “sometimes” fits the description (0); “fitted the description in the past” (1); or “fits the description most of the time” (2). The scores for each item are summed obtaining a total score, with higher score indicating worse behavior. In the present study, the ITSC at 12 months included a total of 28 items (mean score = 2.73; SD = 3.41;