Phthalates and bisphenol: A biomonitoring in Italian mother-child pairs: link between exposure and juvenile diseases

Background

Phthalates (Diethylhexyl) phthalate (DEHP) (in particular) and Bisphenol A (BPA), extensively used as plasticizers, are non-persistent environmental contaminants whose well-documented presence in humans raises concern since they act as endocrine disruptors (EDs), thus potentially affecting human health. EDs exposure during childhood deserves special attention since this represents a crucial susceptible phase of development.

Recent studies have associated the exposure to DEHP and BPA to endocrine-related multifactorial diseases in children, in particular precocious puberty and obesity.

BPA acts as weak agonist of estrogen receptor, interfering with thyroid hormone and with the PPARy (peroxisome proliferator-activated receptor gamma); phthalates are antiandrogens and interfere with PPARy.

Main objectives

• To estimate internal levels of DEHP and its metabolites and BPA in children and adolescents of Italian population
• To investigate the relationship among DEHP and its metabolites and/or BPA internal levels and endocrine related multi-factorial diseases in children
• To evaluate clear cause- effect due to BPA/DEHP exposure in experimental conditions by a juvenile toxicity study in rodents, as innovative animal model for childhood diseases

Main actions

Human biomonitoring study:

• Division of the Italian territory macro areas (North, Centre, South) and each macro area into urban and rural areas
• Involvement and training of pediatricians from the National Health System
• Enrollment of mother-child/adolescent pairs by age and gender; administration of informed consent, questionnaires on lifestyle/food habits; filling in the food diary two days before urine sampling; collection of urine samples
• Analysis of the levels of BPA and DEHP/DEHP metabolites in urine samples

Case-control studies (Rome):

1) idiopathic premature thelarche: enrollment of 30 girls aged 2-7 years
2) idiopathic central precocious puberty: enrollment of 30 girls aged 2-7 years
3) idiopathic childhood obesity: enrollment of 30 boys and 30 girls aged 6-10 years.

Control groups consisting of an equal number of boys and girls of corresponding age will be enrolled for each study.

Urine sampling for analysis of the BPA and DEHP/DEHP metabolites levels.

Blood sampling for analysis of relevant toxicological biomarkers (hormones, lipid and glucose metabolism, liver and thyroid function).

Juvenile rodent toxicological study (corresponding to the same life stages of children in biomonitoring study):

Rats will be exposed, from weaning until sexual maturity, to BPA and DEHP alone and/or in mixtures at doses levels recorded in children population. Levels of thyroid and reproductive biomarkers will be measured in serum. At sacrifice, histopathological analysis on spleen, uterus, ovaries, testes, liver, pancreas, thyroid and adrenal glands will be performed.

Gene expression of hypothalamic-pituitary-gonadal axis factors will be analyzed.

Expected results

• Setting of reference ranges for DEHP and BPA exposure in Italian children and women, according to the area of residence
• Evaluation of environmental factors, food habits and lifestyles as sources of EDs exposure
• Potential correlation between EDs exposure and premature thelarche/precocious puberty/children obesity
• Correlation between biomarkers of exposure and effect

What are endocrine disruptors?

Endocrine disruptors (EDs) are “exogenous substance or mixture, that alter function(s) of the endocrine system, causing adverse health effects in an intact organism, or its progeny, or (sub)populations” (WHO/PCS /2002). The “Community strategy for endocrine disruptors’ (CDM/1999/706) is focused on short, medium and long-term actions to support evaluation, identification and assessment of EDs. Phthalates and BPA are EDs: less toxic compared to pesticides and dioxins but commonly detected both in the environment and in food and, as a consequence, in humans.

Sources of exposure

Plasticizers are additives used to improve the physical and mechanical properties of plastics. BPA improves transparency, mechanical and heat resistance of polycarbonate plastic; it is used in the production of epoxy-phenolic resins for non-food (e.g., thermal paper), as well as for food applications (e.g., food containers). DEHP is used to enhance polymer product flexibility and softness; it is used in some food packaging (e.g., plastic wrap/film), cosmetics, and medical equipment (e.g., blood bags).

Human Biomonitoring Study

Number of participants involved per Macro Area

<table>
<thead>
<tr>
<th>Area</th>
<th>Urban areas</th>
<th>Rural areas</th>
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</thead>
<tbody>
<tr>
<td>NORTH</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>CENTRE</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td>SOUTH</td>
<td>21</td>
<td>13</td>
</tr>
</tbody>
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Enrolled children and mother

- 60 boys and 60 girls

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<thead>
<tr>
<th>Age</th>
<th>Area</th>
<th>Macro Area</th>
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</thead>
<tbody>
<tr>
<td>4-6 year-old</td>
<td>Urban</td>
<td>North</td>
</tr>
<tr>
<td>7-10 year-old</td>
<td>Rural</td>
<td>Centre</td>
</tr>
<tr>
<td>11-14 year-old</td>
<td>Rural</td>
<td>South</td>
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Total: 2160 mother-child pairs

Case-control studies

<table>
<thead>
<tr>
<th>Subjects</th>
<th>BPA</th>
<th>DEHP</th>
<th>MIX</th>
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<tbody>
<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>31</td>
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<tr>
<td>30-10</td>
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Juvenile rodent study

Rats treated in the last for 28 days from weaning (approx. post-natal day 23) until sexual maturity (approx. post-natal day 60).

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