Obtained from: file:///C:/Users/Darko/Downloads/CORDIS\_result\_153420\_en.pdf

***Chemicals:***

PCB153 as a marker of non-dioxin like PCBs,

organochlorine pesticides (DDE and HCB),

brominated flame retardants (BDE47, BDE99 and HBCD),

dioxins (PCDD/Fs and dioxin-like activity measured with CALUX),

perfluorinated compounds (PFOS and PFOA) and the

secondary metabolites of the phthalate DEHP

A total of 5 long term animal studies were carried out in OBELIX. The selected test EDCs were BPA, PFOA, TCDD, DEHP and PCB 153.

**Risk assessment**

One of the main goals of OBELIX is to ascertain if there are risks for obesity or related metabolic disorders in children at current background levels of EDCs. To this end, we performed risk assessment using the 5 EDCs that were tested in animal studies in WP5. Two of the **OBELIX EDCs, namely DDE and BDE-47, were not included in this risk assessment exercise, as we did not perform animal studies on these compounds in OBELIX**. It should be mentioned for DDE, however, that a consistent set of observations was found in WP1 indicating that prenatal DDE is associated with accelerated growth in early childhood, and increased BMI and serum leptin levels at age 6 years. This would suggest that current background levels of DDE are not without risk and further examination of health risks of DDE exposure in children is warranted. To our knowledge there are very limited animal experiments in the literature that examine perinatal exposure to DDE and latent effects on metabolism and energy balance. This is an important knowledge gap that should be studied in the future.

**Explanation of the risk assessment, potentially added to the application**

For the risk assessment, we selected the most sensitive critical effect observed in OBELIX animal studies and compared this to benchmark dose levels (BMDL) established by EFSA. We then calculated a corresponding tolerable daily intake (TDI) level and compared this to exposure and/or body burden (BB) levels reported by EFSA, taking important exposure routes into account. Where possible we also used calculated food exposure data from WP3, in which food frequency questionnaires (FFQ) from FLEHS, Michalovce and LINC were used to calculate exposure to EDCs through the diet.

In summary, our risk assessment of BPA indicated that the OBELIX BMDL05 for decrease in fat pad weight in female offspring (233 µg/kg bw/d) is >10 times smaller than the most sensitive BMDL10 (Benchmark dose level 10) recently proposed by EFSA in 2014 (EFSA 2014). Draft Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). Using the uncertainty factors (UF) recently proposed by EFSA, this OBELIX BMDL would lead to a TDI of 0.28 µg/kg bw/day, which is considerably lower than the current proposed TDI of 5 µg/kg bw/day. This level is above the estimated human lifetime exposure level of up to 0.132 µg/kg bw/day (EFSA, 2014), however, when taking into account combined exposure through diet and other sources such as thermal paper, BPA exposure may exceed this OBELIX TDI for sensitive groups, indicating potential risk of metabolic disruption in humans at background exposure to BPA.

For PFOA, the OBELIX BMDL05 for decrease in fat pad weight in female offspring was also lower than the most sensitive BMDL10 that was proposed by EFSA to derive a TDI. (EFSA 2008. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. Scientific Opinion of the Panel on Contaminants in the Food chain. The EFSA Journal(2008) 653, 1-131.). Applying the same UF as applied by EFSA, the BMDL for decreased fat pad weights in female F1 mice observed in OBELIX would lead to a corresponding TDI value of 0.23-0.28 µg/kg bw/day. Regarding PFOA exposure, the EFSA indicative estimate from food and water is 2 ng/kg bw/day, which correspond very well with the intakes estimated for the OBELIX cohorts FLESH, Michalovce and LINC calculated in WP3. Although OBELIX data indicate a lower TDI than derived by EFSA, it should be mentioned that human exposure may be considerably lower than the TDI based on the OBELIX animal study. EFSA estimated a human exposure of 0.002-0.006 µg/kg bw/day4, which would indicate a indicate P age 8 o f 12 Research and Innovation a sufficient margin of safety.

For TCDD, the most sensitive effect observed in the OBELIX animal study was a decrease in fat pad weight in male F1 mice, corresponding with a BMDL05 which is well above the dose used to establish the current TDI (SCF, 2001. Opinion of the Scientific Committee of Food on the risk assessment of dioxins and dioxin-like PCBs in food. Scientific Committee of Food CS/CNTM/DIOXIN/20 final). This would indicate limited risk of TCDD for metabolic disrupting effects. However, we think human exposure and the results of the epidemiological studies in WP1 should be considered as well in the risk assessment of TCDD. In the OBELIX cohorts, median TEQ levels in blood (FLEHS) and breast milk (Michalovce) ranged from 4.3 to 23.5 pg CALUX-TEQ/g lipid. Assuming a BB equilibrium and a fat percentage of 20%, these values could be calculated into median BB estimates of 0.86 to 4.7 ng CALUX-TEQ/kg bw. Applying the same UF as used by SCF (2001)4 to calculate the LOAEL-EHDI into a TDI value, the LOAEL-BB for TCDD of 40 ng/kg bw in rat derived for the study of Faqi et al. (Faqi, A.S., Dalsenter, P.R., Merker, H.-J., and Chahoud, I. (1998). Reproductive toxicity and tissue concentrations of low doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin in male offspring rats exposed throughout pregnancy and lactation. Toxicology and Applied Pharmacology, 150, 383-392.) can be recalculated into a tolerable human BB of 4 ng/kg bw. Comparing this tolerable human BB to the BB estimates for the OBELIX cohorts demonstrates that human exposure is in the same range as critical exposure levels, or even exceeding these critical levels.

For PCB 153, the most sensitive effect observed in the OBELIX animal study was an increase in glucagon levels in female F1 mice, corresponding with a BMDL05 that is larger than the overall NOAEL range for non-dioxin-like (NDL) PCBs proposed by EFSA (EFSA 2005a. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the commission related to the presence of non dioxin-like polychlorinated biphenyls (PCB) in feed and food. The EFSA Journal (2005) 284, 1 – 137). For NDL-PCBs, EFSA (2005a) estimated an average dietary intake of 10-45 ng/kg bw/day, which is well below the critical effect concentration in OBELIX. However, a “margin of body burden” (MoBB) approach has been used by EFSA for PCB 153 risk assessment in which the NOAEL estimated animal BB is divided by the estimated median human body BB. For the OBELIX mouse study, lipid-based PCB-153 concentrations were used to calculate the serum concentration corresponding to the BMDL05. This serum concentration could be translated into a BB of 1042 µg/kg bw, which is actually lower than the critical BB of 1200 µg/kg bw for PCB-153 in rats. In the OBELIX cohorts, median PCB-153 levels in cord plasma (FLEHS, Michalovce, LINC), maternal serum (Michalovce), or breast milk (HUMIS, LINC) ranged from 0.028 to 0.137 µg/g lipid. These serum levels were calculated into BB values and indicated limited risk with a MoBB of 39-189. Interestingly, although the MoBB estimates for PCB 153 seem large enough to be protective, significant associations were found in OBELIX for background PCB 153 exposure and health effects in children. For example, decreased leptin blood levels have been found in children from the Michalovce cohort at age 7 years that correlate with PCB-153 blood levels at age 6 years (Palkovicova et al, in prep). By applying a benchmark approach to these data, a BMCL of around 0.2 µg/g lipids could be estimated as a critical internal PCB-153 dose. Assuming a fat percentage of 20%, this value can be calculated into a critical BB of 40 µg/kg bw. This value is much lower than the critical BB established in the animal studies performed in OBELIX or reviewed by EFSA (2005a). It is clear that epidemiological data generated in OBELIX should be considered in risk assessment of EDCs, as the use of animal data only to set critical effect levels may underestimate risk in humans.

For DEHP, the most sensitive effect observed in the OBELIX animal study was an increased level of free fatty acids in male F1 mice resulting in a BMDL slightly smaller than the most sensitive NOAEL of 5000 µg/kg bw/day that is proposed by EFSA to derive a TDI (EFSA 2005b. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to Bis(2-ethylhexyl)phthalate (DEHP) for use in food contact materials. The EFSA Journal (2005) 243, 1-20). Applying the same UF as applied by EFSA (2005b), the BMDL in OBELIX would lead to a corresponding TDI value of 44 µg/kg bw/day, which is slightly lower than the TDI of 50 µg/kg bw/day derived by EFSA. Examination of exposure data indicated that external exposure values are lower but in the same range as the TDI derived by EFSA (2005b) or derived from the OBELIX animal study. However, improved exposure assessment of DEHP is recommended, in particular in situations of high neonatal exposures