RESEARCH REPORT - No. 1002 FY 2010-2012

Title of research project	Study on offspring reproductive and developmental effects of fetal and lactational exposure to di(2-ethylhexyl)phthalate and the rrisk assessment
Research project no.	(1002)
Research period	FY 2010–2012
Name of principal research Investigator（PI）	Tamie Nasu

【Abstract】

Objectives: We investigated the effects of di(2-ethylhexyl)phthalate (DEHP) exposure at fetal and lactational periods on offspring development of mice and the mechanism.

Methods: Pregnant mice of Sv/129 wild-type, peroxisome proliferator-activated receptor (PPAR)α-null mice and humanized PPARα (hPPARα) mice were treated with diets containing 0, 0.01, 0.05 or 0.1% DEHP until Post-natal day 21. Some of offspring in each dose was respectively dissected on gestational day 18 (fetuses), postnatal day (PND)2 and PND21 together with mothers. Remaining offspring in dose of 0 and 0.05% DEHP was weaned and divided into two groups on PND21, and then fed commercial control diet and high fat diet, respectively, for 8 weeks (11-week-offspring).

Results and Discussion/Conclusion: Fetal and lactational exposure to DEHP showed the development-dependent effects on the offspring.

Fetal and lactation periods: DEHP decreased the number of live fetuses and PND2 pups, while increased embryo resorption in wild-type and hPPARα mice.

Lactation and postweaning periods: Food consumption at the postweaning period was increased in wild-type and hPPARα offspring exposed to 0.05% DEHP in fetal and lactation periods, but not in PPARα-null one. However, no such influence was observed in high fat diet group. DEHP decreased plasma leptin levels in PND2 and PND21 wild-type offspring, but not in 11-week-offspring. Since exposure to DEHP during lactation period alone did not influence the plasma levels of PND21 offspring, DEHP exposure in fetal period probably decreased the leptin level in PND2 and PND21wild-type offspring, which may be related to the increase in the food consumption in the offspring. In conclusion, DEHP exposure in fetal period may PPARα-dependently influence the feeding behavior in the developmental stage of offspring. In this case, plasma levels of leptin may be important as the marker, though the mechanism of feeding regulation in hPPARα offspring was still unknown. In the testis of PND21 offspring, fetal and lactational exposure to DEHP increased apoptosis of sperm cells irrespective of PPARα genotype.

No influence of DEHP exposure in fetal and lactational periods was observed in methylation of PPARα promotor area of 11-week-offspring.

Mature stage: Exposure with 0.05% DEHP in fetal and lactational periods also decreased plasma testosterone

levels of wild-type 11-week-offspring. In contrast, the exposure increased the numbers of sertoli cell vacuolation of the offspring when they took high fat diet during postweaning periods. No such changes were noted in PPARα-null and hPPARα 11-week-offspring. Exposure with 0.05% DEHP in fetal and lactational periods increased liver triglyceride levels only in PPARα-null mice.

Maternal and paternal age: DEHP exposure of pregnant mice decreased not only plasma triglyceride (TG) but also some fatty acid levels in the maternal plasma of wild-type mice, but not in PPARα-null and hPPARα mice. influence of DEHP neither on testis nor in ovarian of the adult mice were observed. DEHP exposure could not influence liver TG and fatty acid levels in any offspring.

Species and individual differences: In order to assess the species and individual differences in the metabolism of DEHP, hepatic enzyme activities of thirty-eight subjects of various ages and eight 129/Sv male mice were measured. Microsomal lipase activity, measured by the rate of mono(2-ethylhexyl)phthalate (MEHP) formation from DEHP, was significantly smaller in humans than in mice. The Vmax/Km value in humans was a seventh of that in mice, microsomal UDP-glucronocyltranspherase (UGT) activity in human was a sixth of that in mice, and cytosolic aldehyde dehydrogenase (ALDH) for 2-ethylhexanal in humans was one-half.
In contrast, alcohol dehydrogenase (ADH) activity for 2-ethylhexanol (2-EH) was 2.0-fold higher in humans than that in mice. Total amount of DEHP urinary metabolites and MEHP concentration were much higher in intact mice than U.S. general population,as was reported elsewhere, Though DEHP intake was estimated to be similar between these mice and the human population.However, mono(2-ethyl-5-oxo-hexyl)phthalate (5oxo-MEHP) and mono(2-ethyl-5-carboxypentyl)phthalate (5cx-MEPP) levels were higher In the human population than in these mice Human individual difference between all of the DEHP metabolizing enzymes were 10-26 fold, but no such differences were observed among race, sex, age and lifestyle habits. Taken together, the inter-individual difference in metabolism of DEHP in human may be greater than the species difference between mice and human, which may be also involved in the risk assessment of DEHP in addition to the species difference. In the risk assessment of DEHP using the toxicity data of mice, 10-fold uncertainty factor may be appropriate rather than 2.5, and inter-individual difference in the metabolism may be more than 10-fold.