No Observed Adverse Effect Levels of Phthalate Esters on Reproductive and Developmental Toxicity, the Differences with Age and Species in Testicular Toxicity, and Tolerable Daily Intake of DEHP

(Received December 7, 2000)
(Accepted January 23, 2001)

Mutsuko Koizumi\textsuperscript{a)}, Makoto Ema\textsuperscript{b)}, Akihiko Hirose\textsuperscript{a)}, Yuji Kurokawa\textsuperscript{a)} and Ryuichi Hasegawa\textsuperscript{a)}

\textsuperscript{a)} Biological Safety Research Center, National Institute of Health Sciences
\textsuperscript{b)} Division of Biological Evaluation, National Institute of Health Sciences Osaka Branch

Keywords: Phthalate esters, No observed adverse effect level, Difference with species, Di(2-ethylhexyl) phthalate, Tolerable daily intake

Abstract

It is well known that some species of phthalate esters have reproductive and developmental toxicity, monoesters being active metabolites. In June 2000, the Japanese Government established a tolerable daily intake (TDI) for di(2-ethylhexyl) phthalate (DEHP) based on data for testicular and reproductive toxicity because of the report that certain cooked foods had been relatively high contaminated, caused by the use of polyvinyl chloride gloves containing DEHP in the final stage of food packing. In the present paper, first of all, no observed adverse effect levels (NOAELs) or lowest observed adverse effect levels (LOAELs) of various kinds of phthalate esters in terms of reproductive and developmental toxicity endpoints, and the differences with age and species in testicular toxicity are examined and summarized. Then the rationale for establishing a TDI for DEHP and the exposure problem are discussed. This article also includes new information from a draft of the Toxicological Profile for DEHP published in September 2000 by the US Agency for Toxic Substances and Disease Registry, and assessment reports made available in October 2000 from the Center for the Evaluation of Risks to Human Reproduction, US National Toxicology Program.

1. Structurally related toxic potential of phthalate esters

As most studies on reproductive and developmental toxicity of phthalate esters have been conducted using rodents, the NOAELs or LOAELs in rodents (rats and mice) were identified.
a. Effects on male reproductive organs

As rats of weaning age are reported to be sensitive to testicular toxicity, study data from experiments conducted in 4 to 6 week old rats were collected and evaluated. Phthalate esters having straight chain lengths from C4 to C6, such as di-n-butyl phthalate (DBP), di-n-pentyl phthalate (DPP) and di-n-hexyl phthalate (DHP) show clear testicular toxicity. Other phthalate esters having shorter or longer carbon chains such as dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-propyl phthalate (DPrP), di-n-heptyl phthalate (DHpP) and di-n-octyl phthalate (DOP) exert much less or almost no testicular toxicity. The branched phthalate ester, DEHP, also demonstrates testicular toxicity and has the lowest NOAEL of 3.7 mg/kg/day among phthalate esters, probably because of the C4 or C6 structure in its side chain. n-Butyl benzyl phthalate (BBP) shows testicular toxicity because mono-n-butyl phthalate (MBP), the active metabolite of DBP is likely produced by intestinal hydrolysis.

b. Effects on female reproductive organs

DEHP induces elongation of the estrous cycle, obstruction of ovulation and loss of corpora lutea only at a high dose, such as more than 2,000 mg/kg/day. DBP shows no effects including morphological changes in the ovary, even at high doses.

c. Reproductive toxicity

In one- or two-generation studies, C4 to C6 phthalate esters such as DBP, DPP and DHP induced low fertility and decreased number of litters per pair and live pups per litter but the NOAELs were not established. DEHP also exert the same adverse effects with a NOAEL of 14 mg/kg/day. The longer chain phthalate esters, DOP, di-iso-nonyl phthalate (DINP) and de-iso-decyl phthalate (DIDP) demonstrate no reproductive toxicity.

d. Developmental toxicity

With the administration during the organogenetic period, NOAELs of 44, 500 and 500 mg/kg/day for DEHP, DBP and BBP were concluded on the basis of fetal toxicity, causing low fetal viability and body weight reduction, and/or teratogenicity. However, in a two-generation study DBP and BBP were found to cause lowering of body weights of infants at the lower doses of 80 mg/kg/day and 100 mg/kg/day, respectively. Furthermore, NOAELs for branched long chain phthalate esters have been shown to be relatively low, such as 100 mg/kg/day for DINP, causing increased skeletal variation, and 38 mg/kg/day for DIDP, based on low growth of neonates.

High dose administration of DBP, DEHP, BBP and DINP at more than 500 mg/kg/day from late pregnancy to 3 days after birth resulted in morphological adverse effects on male reproductive organs. The only NOAEL established was 50 mg/kg/day for DBP.
2. Age- and Species-dependent testicular toxicity of phthalate esters

a. Age-dependent testicular toxicity of phthalate esters in rats

DEHP induces severe testicular damages in 4 week old rats but not in those 15 weeks of age. The plasma concentration of mono(2-ethylhexyl) phthalate (MEHP), the active metabolite, is higher in younger rats, suggesting higher hydrolysis of DEHP in the intestine of young animals. A co-cultivation study with Sertoli cells and germ cells prepared from rats of various ages indicated the higher sensitivity to MEHP of preparations from younger animals. These two differences might explain the age-dependence of testicular toxicity due to phthalate esters in rats.

b. Species differences in testicular toxicity

Rats and mice are much more sensitive to DEHP than hamsters. Oral administration of MEHP also resulted in higher testicular toxicity in rats than hamsters and incubation of DEHP with intestinal contents from the two species demonstrated 4 times higher hydrolytic activity in rats. Co-cultivation studies with Sertoli cells and germ cells also showed higher sensitivity to MEHP with rats. These results suggest that the difference between rats and hamsters is caused by the higher hydrolytic activity toward DEHP and sensitivity to MEHP in the rats. Similarly, the lack of any testicular toxicity including effects on blood testosterone concentration of DEHP in the marmoset and cynomolgus monkey is speculated to be due to low hydrolytic activity toward phthalate ester in the primate intestine. There is no information on MEHP sensitivity in these species.

3. Establishment of a TDI for DEHP and safety assessment

a. Information on human health

There is only one study reported, demonstrating a correlation between premature breast development in Puerto Rico females of 2 to 3 years old and the blood DEHP content. As for related information, there are two reports on DBP whose toxicity is similar to DEHP in rodent study. A Russian occupational study indicated a decrease in the frequency of pregnancy and births for DBP-exposed workers but the data reliability is questionable. On the other hand, no co-relationship between DBP concentration in semen and the sperm count was shown.

b. Establishment of a TDI and Government Action

Although DEHP is unlikely to be as toxic to the testes of humans at the same level as in rats, the possibility of such testicular toxicity can not be excluded at present. Therefore, the Japanese Government has established a TDI range for DEHP as 40 to
140 \mu g/kg/day from NOAELs of 3.7 and 14 mg/kg/day, based on testicular and reproductive toxicities, respectively. In Japan, extremely high contents of DEHP were recently found in certain cooked foods and the average daily intake in the worst case scenario was estimated to be 35.4 \mu g/kg/day. Therefore the daily intake of DEHP in certain cases could have been within this TDI range. However, daily intake should now remain sufficiently below TDI because the Government has strongly recommended avoiding the use of polyvinyl chloride gloves containing DEHP by workers involved in food treatment.

A specific TDI value may be specified when mechanisms explaining the lack of testicular toxicity in primates are clarified. However, exposure levels to any environmental chemical should always be set as low as possible because safety in humans can not be definitely confirmed solely on the basis of experimental evidence.

Corresponding author:
Mutsuko Koizumi, Division of Risk Assessment, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan