This is a provisional English translation of an excerpt from the original full report.

Risk Assessment Report
Bis(2-ethylhexyl)phthalate (DEHP)

(Apparatuses, Containers and Packages)

Food Safety Commission of Japan (FSCJ)
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ABSTRACT

FSCJ conducted a risk assessment of bis(2-ethylhexyl)phthalate (DEHP) (CAS No.117-81-7) as a substance related to revision of the standards and criteria for apparatuses, containers and packages.
The data used in the assessment are on; acute toxicity (rats, mice and rabbits), subacute toxicity (rats and monkeys), carcinogenic and chronic toxicity (rats and mice), reproductive and developmental toxicity (rats, mice, monkeys and pigs), genotoxicity and epidemiological studies.

The primary adverse effects of DEHP were observed on reproductive and developmental toxicity and carcinogenicity in the tested animals. For reproductive and developmental toxicity, DEHP affected the reproductive system in both male and female rodents. Particulary, maternal exposure to DEHP during gestatimal and lactatimal periods, even with relatively low doses, was reported to result in adverse effects on the reproductive tract in male offspring. Although influences on reproductive and developmental systems are observed in humans, only limited numbers of epidemiological reports are currently available. Therefore, FSCJ estimated the dose-response relationship from experimental animal data but not from the currently available human data.

For carcinogenicity, DEHP has been reported to induce hepatic tumors in mice and rats, but the carcinogenicity in humans is unclear after oral exposure.

With regard to the genotoxicity of DEHP, most of data *in vitro* and *in vivo* were negative. DEHP and its metabolites were thus likely to interact indirectly with DNA. Consequently, FSCJ considered that DEHP and its metabolites exert their effects through epigenetic mechanism rather than their direct actions to DNA.

Hence, FSCJ regarded it as possible to establish the tolerable daily intake (TDI) of DEHP.

The lowest no-observed-adverse-effect level (NOAEL) of all the tests is 3 mg/kg body weight/day, which was obtained in gavage administration study in rats during the period from gestation day 7 to postnatal day 16. FSCJ established a TDI of DEHP of 0.03 mg/kg body weight/day, applying uncertainty factor of 100, which consists of 10 for species difference and 10 for individual difference, to the lowest NOAEL of 3 mg/kg body weight/day.

Risk Assessment

Phthalates such as DEHP are chemical substances widely used as plasticizers in manufacturing plastics, especially for polyvinyl chloride (PVC) products. DEHP may occur in air, soil, water, and food, as a result of being leached, migrated, or sublimated from PVC products.

1. Toxicokinetics

After ingestion in rodents, DEHP is hydrolyzed into MEHP (mono(2-ethylhexyl) phthalate) and 2-EH (2-ethylhexanol) by lipase in the digestive tract prior to absorption. At a dose of up to 200 mg/kg body weight, ca. 50% of the dose was absorbed in primates including humans and in rats. Some reports, however, showed reduced rates (20~25%) of absorption in human's digestive tracts. DEHP and its metabolites are distributed throughout the body and their concentrations are particularly high in the liver, testes, and adipose tissues. No evidence of their accumulation, however, is reported. In humans and rodents, DEHP and its metabolites are secreted in milk and permeate placenta. MEHP is biotransformed into a variety of oxidative metabolites. In rodents, the activation of peroxisome proliferator-activated receptor-α (PPARα) enhances a group of enzyme activities such as CYP4A, which catalyzes the oxidation of MEHP. MEHP and its oxidative metabolites are glucuronidated and then excreted in urine.

In the above-mentioned toxicokinetics of DEHP, the species difference has been reported, especially for metabolism between rodents and humans.

The lipase activity as well as the enzyme induction through PPARα, is lower in humans than in rodents. Comparison of the overall metabolic activity estimated as sum of individual steps of metabolism using toxicokinetics data on the blood and urine, showed the difference in the metabolic ratios between rodents and humans. However, the rapid disappearance of DEHP from the blood, and the higher excretion ratio of oxidative metabolites of MEHP than itself in urine were consistent between humans and rodents. Thus, the lipase activity is unlikely to be a rate-limiting step for DEHP metabolism and the cause of the species difference of metabolic activity between rodents and humans. Oxidative metabolism of MEHP in humans suggests the presence of the ω-oxidating enzyme in humans. Ratios of glucuronide metabolites were higher in human urine than in the rodents. Clear individual differences are reported in their metabolic capacities in humans.

DEHP taken from normal living environments are metabolically cleared well in humans, although metabolic capacities are considered to be slightly higher in rodents than in humans due to the rodent-selective activation through PPARα at the higher exposure level to DEHP. The overall metabolic capacity was considered not so different between humans and rodents, if considered the individual difference in humans. Thus species differences are not taken in the estimation of toxicokinetics.

2. Toxicity

In order to evaluate the effect of DEHP on human health, FSCJ thoroughly reviewed the results of a variety of animal tests and epidemiological findings in humans. Consequently, primary toxicity of DEHP was found to appear as reproductive and developmental toxicity and carcinogenicity in animals.

With regard to the genotoxicity of DEHP, most of the *in vitro* data were negative and the most of data *in vivo* were also negative barring a few positive data. Taken altogether, DEHP and its metabolites were not considered to interact directly with DNA. Consequently, FSCJ reached the consensus that DEHP and its metabolites may have epigenetic effects, but they are not classic genotoxic chemicalss.

(1) Carcinogenicity

Oral administration of DEHP has been shown to induce liver tumors in mice and rats. The lowest NOAEL obtained from tests on carcinogenicity of DEHP was 28.9 mg/kg body weight/day for hepatocarcinogenesis, which was obtained in a 104-week dietary study in rats[1,2]. However, potential carcinogenicity of DEHP in humans through oral exposure is unclear due to the scarcity of available data. Although there was a study showing no correlation between occupational exposure through inhalation and carcinogenicity, the limited number of the objects and the low exposure level precluded the use for the assessment of the carcinogenicity in humans.

Hepatocarcinogenesis in rodents has been considered to be linked primarily through PPARα, while clear species difference of its activity has been known between rodents and humans. Recent reports have also shown that administration of DEHP induces liver tumors even in Pparα-knockout mice, and also that constitutive androstane receptor (CAR) as well as PPARα is involved in DEHP-induced carcinogenesis in rodents. Thus, several mechanisms have been suggested for DEHP carcinogenicity.

In 2000, International Agency for Research on Cancer (IARC) evaluated DEHP and classified it into Group 3, where the item is not classifiable as to its carcinogenicity to humans. However, IARC reevaluated DEHP in 2011 and classified it into Group 2B, where the item is potentially carcinogenic to humans.

(2) Reproductive and Developmental Toxicity

Concerning reproductive and developmental toxicity, DEHP has been reported to affect the reproductive system in male and female rodents. Particularly, the exposure of the embryos and offspring to relatively low levels of DEHP through the dams during the gestation and lactation stages induces adverse effects on reproductive tracts in male offspring.

Regarding reproductive toxicity, various mechanisms including anti-androgenic effects have been proposed, but not yet proven. Mechanism for developmental toxicity of DEHP is also still unclear, although the involvement of PPARα has been suggested.

Studies on a dose-response relationship for reproductive and developmental toxicity in test animals have reported adverse effects on male reproductive tracts at a dose of about 10 mg/kg body weight/day. The lowest NOAEL of all these results was obtained in a gavage administration study in rats during the period from gestation day 7 to postnatal day 16[3]. The NOAEL and lowest-observed-adverse-effect level (LOAEL) established based on a reduction in anogenital distance (AGD) and in reproductive organ weights in male offspring were 3 mg/kg body weight/day and 10 mg/kg body weight/day, respectively.

Human epidemiological studies are now focusing to a specific adverse end point and reporting rather consistent results which are observed also in rodents. Some studies in general populations in the United States and in Japan reported a correlation between an increase in urinary concentration of DEHP metabolites in pregnant women and an AGD reduction observed in male children born from them. A correlation between the urinary concentration of DEHP metabolites in adult men and changes in sex hormones in their blood have also been detected. DEHP intakes estimated from urinary concentrations of DEHP metabolites suggest that humans have higher susceptibility than animals.

3. Establishing of TDI

While the carcinogenicity of DEHP has been identified in rodents, FSCJ considered that DEHP may exert the effect through epigenetic mechanism, but not classical genotoxic mechanism.

Hence, FSCJ concluded that the tolerable daily intake (TDI) of DEHP can be established.

In rodents a PPARα-mediated pathway has been considered to be the primary mechanism of hepatocarcinogenesis. Since PPARα-mediated process shows a clear species difference between rodents and humans, the carcinogenic mechanism is thus difficult to be extrapolated directly into humans from

rodents. Recently, mechanisms through several pathways other than the PPARα-mediated one have also been suggested for the carcinogenicity. However, at present, it is unclear which of those pathways is involved in carcinogenic effects in rodents and how such pathway works in the mechanism. Moreover, it is unclear whether carcinogenic effects via these mechanisms not mediated through PPARα can be directly extrapolated into humans from rodents. Therefore, it is difficult to extrapolate the mechanism for hepatocarcinogenesis in rodents directly into humans. In addition, the carcinogenic effect of DEHP has not been found in humans. For these reasons, in human risk assessment of DEHP, it is difficult to establish the TDI by extrapolating the NOAEL derived from carcinogenic endpoint in rodents into humans.

In contrast, the effects of DEHP on reproductive and developmental systems in humans have been suggested. Therefore, FSCJ considered it appropriate to establish the TDI for DEHP based on the reproductive and developmental effects.

While plural studies in rodents showed DEHP's influence on reproductive tracts in male offspring at similar doses, only few epidemiological studies are available in humans. Therefore, the dose-response relationship can be hardly established using human data on DEHP at the present moment. Consequently, FSCJ considered it appropriate to implement this assessment based on the animal data.

In conclusion, FSCJ established a TDI of 0.03 mg/kg body weight/day by applying uncertainty factor of 100 to the lowest NOAEL of 3 mg/kg body weight/day for reproductive and developmental toxicity from animal studies.

According to recent epidemiological studies, there is a correlation between the low dose exposure of pregnant women to DEHP (10 μg/kg body weight/day or less when converted from urinary concentration of MEHP) and an AGD reduction observed in male children born from them. Although such epidemiological reports are still few in number, these studies are on human effects. Understanding of the actual exposure status to phthalates awaits the further progress of epidemiological studies.

TDI 0.03 mg/kg body weight/day	
(A basis for establishing TDI)	Reproductive and developmental toxicity study
(Animal species)	Rats
(Period)	From gestation day 7 to postnatal day 16
(Dosing method)	Gavage administration
(Findings used as a basis for establishing NOAEL)	Reduction in AGD and reduction in reproductive organ weight in male offspring
(NOAEL)	3 mg/kg body weight/day
(Uncertainty Factor)	100 (Species difference: 10, individual difference: 10)