# Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Material in Contact with Food (AFC) on a request from the Commission related to

# Di-Butylphthalate (DBP) for use in food contact materials

## Question N° EFSA-Q-2003-192

**Adopted on 23 June 2005 by written procedure**

**SUMMARY**

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) has been asked to re-evaluate di-butylphthalate (DBP) for use in the manufacture of food contact materials.

Previously, a temporary Tolerable Daily Intake (t-TDI) of 0.05 mg/kg bw was set by the Scientific Committee for Food (SCF), based on the endpoint of peroxisome proliferation in rodent liver. There is now a scientific consensus that liver peroxisome proliferation in rodents is not relevant for human risk assessment. The critical effects of DBP relate to reproduction. From the several studies available, the critical observations were as follows.

In a two-generation reproduction study in rats with a continuous breeding protocol and with exposure of both male and female animals, the lowest dose-level of 0.1 % in the diet (52 mg/kg bw/day for males and 80 mg/kg bw/day for females) appeared to be a Lowest Observed Adverse Effect Level (LOAEL), based on embryotoxic effects on pup weight and number of live pups per litter. These effects were seen in the absence of maternal toxicity. It should be noted that the LOAEL of 52 mg/ kg bw/day (0.1% in the diet) was derived from an extensive study utilising sensitive endpoints (such as sperm parameters, oestrous cycle characterization and detailed testicular histopathology).

In another reproduction study, a No Observed Adverse Effect Level (NOAEL) of 50 mg/kg bw/day and a LOAEL of 100 mg/kg bw/day for toxicity of DBP on male reproductive development in the F1 generation have been observed.

A recent developmental toxicity study in the rat, with dietary exposure to DBP during the period from late gestation (gestational day 15) to the end of lactation (postnatal day 21), has shown effects on the development of male and female offspring at lower doses than those found previously. Based on loss of germ cell development and mammary gland change at 20 mg/kg in the diet (the lowest tested dose), a NOAEL could not be established.
However, given the reversibility of the effects at all dose levels and especially at the lowest dose level (20 mg/kg feed, which corresponds to 1.5 to 3 mg/kg bw/day) and also given that in several reproductive toxicity studies with longer exposure periods approximately 30 -fold higher NOAELs or LOAELs have been determined, an uncertainty factor of 200, to derive a TDI for DBP based on the LOAEL of 20 mg/kg feed is considered sufficient.

According to the above statement, the Panel allocated a TDI for DBP of 0.01 mg/kg bw, based on a LOAEL of 2 mg/kg bw/day and making use of an uncertainty factor of 200.

The limited available data on DBP concentration in foods and diets in the UK and Denmark were used to provide an estimation of dietary exposure. In the UK, mean and high (97.5th percentile) intakes of DBP from dietary sources were estimated to be respectively 0.013 and 0.031 mg/person/day in the adult population (equivalent to 0.2 and 0.5 μg/kg bw/day) considering a 60 kg adult.
In a Danish study, DBP estimated mean exposure ranged from 0.13 to 0.29 mg/day, i.e. 1.8 to 4.1 μg/kg bw/day, considering a 70 kg adult. Based on the highest concentration of DBP determined, exposure at high percentiles was estimated as 0.72 mg/day equivalent to 10.2 μg/kg bw/day.
In a further Danish study, the main dietary sources of exposure were estimated to be root crops (83%) and leaf crops (13%). The total daily oral intake at the regional level (Denmark) was estimated to be 1.6 μg/kg bw/day in adults, 8 μg/kg bw/day in children aged 1 to 6 years, and 3.5 μg/kg bw/day in children aged 7 to 14 years.
Based on the detection limit, intake from infant formulae would be less than 16.4 μg/kg bw/day in infants of less than 6 months and 6.6 μg/kg bw/day in infants of more than 6 months. For infants of more than 6 months, ready-to-use baby foods were also taken into account and the exposure was therefore estimated as less than 7.9 μg/kg bw/day.

The Panel noted that exposure to DBP from food consumption is in the range of the TDI. There are, however, a number of other sources which contribute to the overall human exposure to DBP. The Panel recommends that improved estimates of exposure to DBP from all sources along with their relative importance should be provided in order to decide what proportion of the TDI can be allocated to food contact materials alone.

**KEY WORDS :**

Di-butylphthalate, DBP, food contact materials, CAS n° 84-74-2, FCM Ref N° 74880.

## BACKGROUND

DBP may be present in food, either due to migration from food contact materials containing DBP or due to its widespread presence as an environmental contaminant which can be found in air, water, soil and food. DBP was evaluated by the Scientific Committee for Food (SCF) in 1988 when a t-TDI for use in food contact materials was established based on the then most sensitive end-point of peroxisome proliferation in rodent liver (SCF, 1995). There is a scientific consensus that liver peroxisome proliferation in rodents is not a relevant endpoint for human risk assessment (IARC, 1995). The Panel has therefore been asked to re-evaluate DBP for use in food contact materials.

## TERMS OF REFERENCE

The Commission asks EFSA to re-evaluate di-butylphthalate (DBP) for use in the manufacture of food contact materials.

## ASSESSMENT

## 1. Chemistry

### Identification of the substance

CAS-No.: 84-74-2

EINECS-No.: 201-557-4

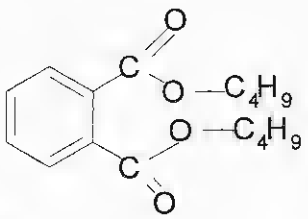
IUPAC name: dibutylphthalate

FCM Ref N° 74880

Synonyms: Di-n-butylphthalate, 1,2-Benzenedicarboxylic acid, dibutyl ester (9CI), Phthalic acid, dibutyl ester (6CI, 8CI), Bis-n-butyl phthalate, Butyl phthalate, DBP, DBP (ester), Di(n-butyl) 1,2-benzenedicarboxylate, Dibutyl o-phthalate, n-Butyl phthalate, Phthalic acid di-n-butyl ester

Molecular formula $C_{16}H_{22}O_4$

Structural formula:



Molecular weight: 278.34

### Purity/impurities, additives

Purity: ≥ 99% (w/w)

Impurity: ca. 0.01% (w/w) butan-1-ol

ca. 0.01% (w/w) butyl benzoate

Additives: none

### Physico-chemical properties

Physical state: oily liquid

Melting point: - 69 °C

Boiling point: 340 °C at 1013 hPa

Relative density: 1.045 g/cm$^3$ at 20 °C

Vapour pressure: $9.7 \pm 3.3 \times 10^{-5}$ hPa at 25 °C

Water solubility: 10 mg/l at 20 °C

Partition coefficient n-octanol/water: log $K_{ow}$ 4.572

## 2. Use

Based on data from industry (1995), an average of around 76% of the total DBP production is used as plasticiser in polymers, 14% in adhesives, 7% in printing inks and the remaining 3% of DBP is used in miscellaneous other applications.

## 3. Exposure via food

DBP is present in a large number of end products, some of which are available for consumer use. A significant use is in the food wrap or food packaging area.

No data on the levels of DBP in food in the EU attributable to migration from food contact materials have been submitted by the industry. Exposure assessment based on analytical determination of the total concentration of DBP in samples of foods or diets allow assessment of an overall dietary exposure from packaging material and other sources.

Several published data on levels of DBP in food were found in the literature. High levels (in the range of 10 to 50 mg/kg food) were reported by Castle *et al.* (1988, 1989) in confectionery and mixed dishes.

An assessment of exposure to phthalates was performed based on the analysis of stored samples from the Total Diet Study conducted in the UK in 1993. Concentration data were combined with food consumption data from the National Diet and Nutrition Study of British Adults. Since phthalates are fat soluble, only those 10 food groups which make a major contribution to dietary fat intakes were selected for analysis. Among these, carcass meat, eggs, poultry and milk were analysed for individual concentrations of phthalates since these four food groups accounted for approximately 85% of the estimated dietary intake of total phthalates. Mean and high (97.5th percentile) intake of DBP from these sources were estimated to be respectively 0.013 and 0.031 mg/person/day in the adult population, equivalent to respectively 0.2 and 0.5 µg/kg bw/day for a 60 kg adult (MAFF, 1996).

In a Danish study (Petersen and Breindahl, 2000), DBP was analysed in 29 different meals collected by test persons during 24 hours. Results were normalised to a daily diet of 10 MJ of energy and to a body weight of 70 kg in order to estimate total daily exposure. Among the 29 samples, 6 were above the limit of determination. The estimated mean concentration of DBP in the diet varied according to the values assigned to the 23 samples which were under the limit of determination. Estimated mean exposure therefore ranged from 0.13 to 0.29 mg/day, i.e. 1.8 to 4.1 µg/kg bw/day, considering a 70 kg adult. Based on the highest concentration of DBP determined, exposure at high percentiles was estimated as 0.72 mg/day equivalent to 10.2 µg/kg bw/day.

A further Danish assessment of DBP total dietary exposure based on estimated and measured concentrations in environmental compartments using the European Union System for the Evaluation of Substances (EUSES, a computer modeling program) was reported recently (Müller *et al*, 2003). The main dietary sources of exposure were estimated to be root crops (83%) and leaf crops (13%). These high contributions of vegetables to oral DBP exposure may indicate that the exposure estimates for the UK, as given above, might be underestimates of the actual exposure because the UK figures were only based on food from animal sources.
For Denmark, the total daily oral intake at the regional level was estimated to be 1.6 µg/kg bw/day in adults, 8 µg/kg bw/day in children aged 1 to 6 years, and 3.5 µg/kg bw/day in children aged 7 to 14 years. The highest local daily oral intake was estimated as 60 µg/kg bw/day in adults, 400 µg/kg bw/day in children aged 1-6 years, and 200 µg/kg bw/day in children aged 7-14 years. It must be

underlined that more than 90% of these maximum exposure values derive from the highest estimated value of exposure via the local environment (printing inks) and consequently are not related to the diet itself. The contribution from dermal and inhalation exposure was negligible. Furthermore, EUSES, the computer modeling program which has been used for these intake estimates is a conservative one and the obtained values, especially for the local daily intakes, are not representative of the possible exposure via food contact materials.
In the same study (Müller *et al*, 2003), exposure from infant formulae was estimated based on two scenarios: an infant of less than 6 months weighing 5.5 kg and ingesting 900 g/day of formulae and an infant of more than 6 months weighing 8 kg and ingesting 525 g/day of formulae. DBP was measured in 11 commercial products and was below the detection limit (0.1 mg/kg of wet weight). Based on this detection limit, intake from infant formulae would be less than 16.4 µg/kg bw/day in the infant of less than 6 months and 6.6 µg/kg bw/day in the infant of more than 6 months. For infants of more than 6 months, exposure from ready-to-use baby foods was also taken into account, considering the daily consumption of one jar of 250 g containing 0.04 mg DBP/kg of wet weight (the maximum measured concentration among 11 commercial products). Total exposure from infant formulae and ready to use baby foods in infants aged more than 6 months was therefore estimated as less than 7.9 µg/kg bw/day.

## 4. Toxicological evaluation

### *Introduction*

The Panel did not carry out a new extensive risk assessment but took cognisance of the previous evaluations by the SCF and in particular considered the more recent DBP European Union Risk Assessment Report (RAR), prepared for the European Union Existing Substances Regulation, 793/93, 2001 (Annex I), and the comments of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) on this RAR (Annex II), in order to determine the most significant toxicological end-point for risk assessment. In addition, pivotal studies available since the RAR was published were included. Based on this information, the Panel focused on the most sensitive toxicological end-points for the evaluation of DBP, taken from the reproduction/developmental toxicity studies with this substance.

The SCF expressed its opinion on DBP in 1988 (SCF, 1995) based on the following statement in the safety data sheet:

> "Reviewing the evaluation on the phthalate esters and comparing the data on DBP with the data on di(2-ethylhexyl)phthalate, the phthalate ester studied most extensively, the Committee decided to use a safety factor of 1000 for calculating the TDI for DBP. This means that a t-TDI of 0.05 mg/kg b.w. is established".

The key toxicological aspects of DBP described in the EU RAR and the CSTEE opinion are summarized below. Further details are given in Annex 1 and 2.

> Based on the data available for DBP from a variety of genotoxicity studies and taking into consideration the non-genotoxic properties of other phthalate esters, the RAR concluded that DBP can be considered as a non-genotoxic substance. No adequate long-term toxicity and/or carcinogenicity studies on DBP are available.
>
> The pivotal study for human risk characterisation was considered to be a two-generation reproduction study in rats with a continuous breeding protocol and with exposure of both male and female animals (NTP, 1995; Wine *et al.*, 1997). The lowest dose-level of 0.1 % in the diet (52 mg/kg bw/day for males and 80 mg/kg bw/day for females) appeared to be a LOAEL based on embryotoxic effects.

In its opinion expressed on 24-4-2001, the CSTEE agreed with the RAR conclusions outlined above and agreed that the male reproductive system is considered to be a main target of DBP toxicity. The CSTEE recommended that the more recent study performed by Mylchreest *et al.* (2000), establishing NOAEL (50 mg/kg bw/day) and LOAEL (100 mg/kg bw/day) values for toxicity of DBP on male reproductive development in the F1 generation as well as the earlier 2-generation rat study (NTP, 1995; Wine *et al.*, 1997) that established a LOAEL of 52 mg/kg bw/day for embryotoxicity in the F2-generation, should be used in the evaluation of the risk of reproductive toxicity.

### *Specific studies considered by the AFC Panel*

The following studies on reproduction and developmental toxicity have been considered by the Panel for the determination of a NOAEL which could be used as a basis for a TDI calculation. Further details on these studies are given in Annex 1 (European Union Risk Assessment Report) and Annex 2 (CSTEE opinion).

In a one-generation reproduction study in mice, the NOAEL was 0.3% in the diet, equivalent to 420 mg/kg bw/day, based on effects on maternal fertility and embryotoxicity (Lamb *et al.*, 1987; Morissey *et al.*, 1989).

In one-generation reproduction studies in rats, in which females and males were exposed separately, NOAELs of 50 mg/kg bw/day in females and 500 mg/kg bw/day in males have been reported (Gray *et al.*, 1999).

In a two-generation reproduction study in rats, a LOAEL (52 mg/kg bw/day for males and 80 mg/kg bw/day for females) based on embryotoxic effects was reported (NTP, 1995; Wine *et al.*, 1997).

In the reproduction study performed by Mylchreest *et al.* (2000), a NOAEL (50 mg/kg bw/day) and LOAEL (100 mg/kg bw/day) for toxicity of DBP on male reproductive development in the F1 generation have been observed.

In a recent developmental toxicity study (Lee *et al.*, 2004) with exposure during the period from late gestation (Gestational day 15) to the end of lactation on postnatal day 21 (PND 21), maternal rats were given DBP at dietary concentrations of 0, 20, 200, 2000 and 10000 mg/kg. Major results of this study are summarised below.

At PND 2, anogenital distance was significantly reduced in 10000 mg/kg male offspring. At PND 14, the incidence of retained nipples/areolae was increased in all treated male offspring compared with controls but the increase was only significant at 10000 mg/kg. At PND 21, in males, reduction of spermatocyte development as manifested by a decreased number of spermatocytes was observed from 20 mg/kg with dose-dependent increased incidence or/and severity. A significant increase in scattered foci of aggregated Leydig cells was observed at 2000 mg/kg and 10000 mg/kg. In the epididymis, significantly decreased ductular cross sections, indicating reduced coiling, were observed at 2000 and 10000 mg/kg. In the mammary glands, dilatation of alveolar buds and/or ducts was seen in male offspring from 20 mg/kg with low incidence but not achieving statistical significance in any group. In female offspring, hypoplasia of the alveolar buds of the mammary glands was observed in animals from 20 mg/kg with a statistically significant increase at 20, 200, 2000 and 10000 mg/kg ($P<0.05$).

At postnatal week 11 (PNW 11), in males, loss of germ cell development was significant at 2000 mg/kg and above. This lesion differed markedly in severity between animals. Significant increases in vacuolar degeneration in the mammary glands of males was present from 20 mg/kg but with similar incidence and qualitative gradation of change across the dose groups.

At PNW 20 and for the 2000 mg/kg dose, the incidence of loss of germ cell development was increased compared to controls, but without statistical significance and the change was minimal. Mammary gland lesions were observed in the untreated animals at this time point, but the incidence and/or qualitative gradation of change were increased in DBP exposed male animals with statistical significance for vacuolar degeneration at 200 mg/kg and for alveolar atrophy at 200 and 2000 mg/kg.

Thus in this study effects were noted at the lowest dose tested of 20mg/kg in the maternal diet (1.5-3.0 mg/kg bw/day). However, the loss of germ cell development was reversible at this dose, but was observed with a clear monotonic dose-dependency from 200 mg/kg (14-28 mg/kg bw/day) to 10000mg/kg (712-1372 mg/kg bw/day) at PNW 11. At PNW 20, the same effect was found but without statistical significance at the 20, 200 and 2000 mg/kg doses. Similarly, effects on the mammary gland were present in the 20 mg/kg males at PND 21 and PNW 11 but there was no significant effect at PNW 20.

## CONCLUSIONS

Based on all the available toxicological evidence, the Panel concludes that effects on reproduction and development are the most sensitive end-points on which to base the risk assessment for DBP. Previous reviews have identified as pivotal several rat reproduction studies conducted in the last decade, which gave NOAELs or LOAELs in the region of 50 mg/kg bw/day, with the critical effect being on male reproductive development.

A recent developmental toxicity study in the rat (Lee *et al.*, 2004), with dietary exposure to DBP during the period from late gestation (gestational day 15) to the end of lactation (postnatal day 21), has shown effects on the development of male and female offspring at lower doses than those found previously, having examined the development of reproductive tissues in considerable detail at various ages postnatally. Reduction of testicular spermatocyte development and mammary gland changes at low incidence in both sexes of offspring were seen at PND 21 at the lowest dose tested of 20 mg/kg (1.5-3.0 mg/kg bw/day) and above, with dose-dependent increased incidence or/and severity. Loss of germ cell development was no longer present at 20 mg/kg at postnatal week 11, but was still present with dose-dependency from 200 mg/kg (14-28 mg/kg bw/day) to 10000 mg/kg (712-1372 mg/kg bw/day). Non-dose-related, but statistically significant effects on the mammary gland persisted to postnatal week 11 in males at all doses, but by postnatal week 20, significant effects were only seen from 200 mg/kg and above. Based on loss of germ cell development and mammary gland changes at 20 mg/kg in the diet (the lowest tested dose), the Panel noted that a NOAEL could not be established. However, given the reversibility of the effects at all dose levels and especially at the lowest dose level (20 mg/kg feed, which corresponds to 1.5 to 3 mg/kg bw/day) and also given that in several reproductive toxicity studies with longer exposure periods only approximately 30-fold higher NOAELs or LOAELs have been determined, a safety factor of 200, to derive a TDI for DBP based on the LOAEL of 20 mg/kg feed from the Lee *et al* (2004) study is considered sufficient.

According to the above statement, the Panel allocated a TDI for DBP of 0.01 mg/kg bw, based on a LOAEL of 2 mg/kg bw/day and making use of an uncertainty factor of 200.

The limited available data on DBP concentration in foods and diets in UK (1993) and Denmark (2003) were used to provide an estimation of dietary exposure. In the UK, mean and high (97.5th percentile) intakes of DBP from dietary sources were estimated to be respectively 0.013 and 0.031 mg/person/day in the adult population (equivalent to 0.2 and 0.5 μg/kg bw/day considering a 60 kg adult).
In a Danish study (Petersen and Breindahl, 2000), the DBP estimated mean exposure ranged from 0.13 to 0.29 mg/day, i.e. 1.8 to 4.1 μg/kg bw/day, considering a 70 kg adult. Based on the highest

concentration of DBP determined, exposure at high percentiles was estimated as 0.72 mg/day equivalent to 10.2 µg/kg bw/day.
In a further Danish study (Müller *et al*, 2003), the main dietary sources of exposure were estimated to be root crops (83%) and leaf crops (13%). The total daily oral intake at the regional level (Denmark) was estimated to be 1.6 µg/kg bw/day in adults, 8 µg/kg bw/day in children aged 1 to 6 years, and 3.5 µg/kg bw/day in children aged 7 to 14 years.
Based on the detection limit, intake from infant formulae would be less than 16.4 µg/kg bw/day in infants of less than 6 months and 6.6 µg/kg bw/day in infants of more than 6 months. For infants of more than 6 months, ready-to-use baby foods were also taken into account and the exposure was therefore estimated as less than 7.9 µg/kg bw/day.

The Panel noted that exposure to DBP from food consumption is in the range of the TDI. There are, however, a number of other sources which contribute to the overall human exposure to DBP. The Panel recommends that improved estimates of exposure to DBP from all sources along with their relative importance should be provided in order to decide what proportion of the TDI can be allocated to food contact materials alone.

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# ANNEX 1

## Extracts from the European Union Risk Assessment Report on DBP
Consolidated Final Report (dated June 2004)

### Summary on mutagenicity and carcinogenicity

In assays detecting gene-mutations in bacteria, only one assay was negative in all 4 strains tested without and with metabolic activation. In two other assays, equivocal (Agarwal *et al.*, 1985) and positive (Seed, 1982).results were seen in strain TA 100 only, without metabolic activation. The positive effects were weak and seen at cytotoxic doses.
A gene-mutation test in yeast cells showed negative results (Zimmermann *et al.*, 1984).
In a mouse lymphoma assay performed only without metabolic activation, gene-mutations were induced at highly cytotoxic concentrations (NTP, 1995). An adequately performed test for gene-mutations in mouse lymphoma cells showed negative effects without metabolic activation; with metabolic activation positive effects were seen (Hazleton, 1986). In the same experiment diethylphthalate showed negative results while it is expected that, based on structure-activity relationships, mutagenic activity would increase with decreasing length of the alkyl chain. Also butylbenzyl-, di(2-ethylhexyl)-, diisononyl- and diisodecylphthalate showed negative results in the same experiment.
No chromosomal aberrations in mammalian cells were seen but the tests were performed without metabolic activation only. In one test also the induction of SCE's was studied and a slight (<2x), but statistically significant increase of SCE's was seen at all three dose-levels, but without any dose-relationship (Abe and Sasaki, 1977).
A micronucleus study performed according to current standards showed negative results (BASF, 1990). In mice exposed for 13 weeks to DBP in their diet no induction of micronuclei was observed either (NTP, 1995).
In conclusion *in vitro* studies gave an indication for a genotoxic effect in one assay, but this effect was not seen with other dialkylphthalates in the same experiment. No genotoxic effects for dibutylphthalate were observed in *in vivo* studies detecting chromosomal aberrations.

Based on the data available for DBP from a variety of genotoxicity studies as described above and taking into consideration the non-genotoxic properties of other phthalate esters, DBP can be considered as a non-genotoxic substance.

No adequate long-term toxicity and/or carcinogenicity studies in animals as well as man are available.

### Summary on toxicity for reproduction and development

Reproduction studies

In an oral reproduction study in CD-1 mice according to a continuous breeding protocol and including the production of one generation, doses of 0.03, 0.3 and 1.0% DBP in the diet (ca. 0, 40, 420 and 1410 mg/kg bw/day) were administered to groups of 20 m and 20 f animals for a 7d premating period, after which the animals were grouped as mating pairs and treated during a 98 day mating period. A control

group of 40 m and 40 f mice received the basal diet. After the 98-days cohabitation period the pairs were separated and exposed during which period any final litters were delivered and kept for at least 21 days. At the end of the continuous breeding period a 7-day crossover mating trial was performed with Fo animals of control and 1% groups. $F_0$ parents showed a significantly decreased groweighth (males only) and significantly increased liver weights (females only) at 1.0% in the diet. At 1.0% in the diet statistically significant decreases in percentage of fertile pairs, no. of litters/pair, no. of live pups/litter and proportion of pups born alive were seen. Lower dose-levels did not cause these effects. Females and not males were affected as was shown in the crossover mating trial. In this trial between control males and 1.0% females statistically significant decreases in percentage of fertile pairs, no. of live pups/litter, proportion of pups born alive and live pup weight were observed. The NOAEL for parental and embryotoxicity is 0.3% in the diet (ca. 420 mg/kg bw/day) in this study (Lamb *et al.*, 1987; Morissey *et al.*, 1989).

Gray *et al.* (1999) performed a multigeneration study in LE hooded rats. Both male and female animals (10-12 animals/sex/group) of only the $P_0$ generation received orally by gavage 0, 250 or 500 mg DBP/kg bw/day from weaning, through puberty, young adulthood, mating and lactation. Another group of only males received 1000 mg/kg bw/day. When the $P_0$ animals were mated, treated animals were paired with untreated controls. $F_1$ animals were not treated. After puberty $F_1$ animals were selected (16/sex/group) for fertility assessment under continuous mating conditions over 11 breeding cycles.
In the $P_0$ generation delayed puberty (preputial separation) was seen in males at all dose-levels. DBP treatment did not accelerate the age at vaginal opening or induce persistent vaginal cornification, effects indicative of subchronic estrogen exposure. The $P_0$ generation showed reduced fertility in male and female animals at 500 and 1000 (males only) mg/kg bw/day. Infertility in males was related to testicular atrophy and reduced sperm production, while treated females cycled and mated sucessfully, but many treated females (500 mg/kg bw/day) aborted their litters around midpregnancy. In the $F_1$ offspring which were exposed only *in utero* and lactational via dams (data only from $F_1$ animals from dams treated with 0, 250 and 500 mg DBP/kg bw/day), urogenital malformations/abnormalities including a low incidence of agenesis of the epididymis, hypospadias, ectopic testis, renal agenesis and uterine abnormalities (partial agenesis or lack of implants in one uterine horn) were seen. In addition a few treated animals displayed anophthalmia. Furthermore $F_1$ males from treated mothers exhibited reduced cauda epididymal sperm numbers. The $F_1$ offspring showed reduced fecundity (significantly fewer $F_2$ pups; number pups/litters 179/24, 76/10, and 20/4 for 0, 250 and 500 mg/kg bw/day, respectively) in similarly treated pairs under continuous breeding conditions. The lowest dose-level of 250 mg/kg bw/day in this study is a LOAEL.

In an other oral reproduction study in Sprague-Dawley rats according to the continuous breeding protocol and including the production of two generations, doses of 0, 0.1, 0.5 and 1.0% in diet (0, 52, 256 and 509 mg/kg bw/day for males and 0, 80, 385 and 794 mg/kg bw/day for females) were administered to groups of 20 m and 20 f animals for a 7day premating period after which the animals were grouped as mating pairs and treated during a 112day cohabitation period. A control group of 40 m and 40 f rats received the basal diet. After the 112day cohabitation period the pairs were separated and exposed during which period any final litters were delivered and kept for at least 21 days. Thereafter treatment of F1 animals was initiated at the same concentration as their parents. At the end of the continuous breeding period also a 7-day crossover mating trial was performed with Fo animals of control and 1% groups. During the continuous breeding phase 1.0% in the diet caused a reduction in groweighth of Fo females. The total number of live pups/litter was statistically significantly decreased at all dose-levels with a dose-relationship. Live pup weights. were significantly decreased at 0.5 and 1.0% in the diet. In the crossover mating trial, designed to determine the affected sex, no effect upon mating, pregnancy or fertility indices were seen. Fo females at 1.0% showed decreased body weights. and increased rel. liver and kidney weights. Fo males at 1.0% revealed increased rel. liver-, kidney-, and right cauda epididymis weights. F1 males at 0.5% showed significantly increased kidney weights. Sperm parameters (sperm concentration and motility, % abnormal sperm or testicular spermatid head count), estrous cyclicity, and estrous cycle were not affected. The weight of pups from treated females (1.0% in the diet) was statistically significantly decreased.

During the continuous breeding phase, after the cross-over mating trial with the F0 parents and at production of the F2 generation mating, pregnancy and fertility indices for F1 parents were statistically significantly lower at 1.0% in the diet. Live F2 pup weights. were statistically significantly lower at all dose-levels (also after adjustment for litter size). Female F1 parents at 1.0% showed statistically significantly lower body weights. and absolute organ weights (right ovary, liver, kidneys). In male F1 parents at 1.0% body weight. and rel. weights. of all reproductive organs were lower while rel. liver and kidney weights. were statistically significantly increased. Epididymal sperm count and testicular spermatid head count were statistically significantly decreased at 1.0%. Epididymides were absent or poorly developed in 12/20 F1 males at 1.0% and in 1/20 F1 males at both lower dosage levels. In 4/20 males at 1.0% and 1/20 at 0.5% in diet testicular atrophy was seen. Testes of 3/20 males at 1.0% were not descended into the scrotal sacs; 4/20 males at this dose-level had poorly developed seminal vesicles and 4/20 had an underdeveloped prepuce or penis. Histopathology showed degeneration of seminiferous tubules in 8/10 F1 males at 1.0% and in 3/10 at 0.5% DBP in the diet. 7/10 F1 males at 1.0% revealed testicular interstitial cell hyperplasia. Histopathology of seminal vesicles revealed in 1/10 F1 males at 1.0% vesiculitis with inspissated secretion. There was no indication of an effect on estrous cyclicity or duration of the estrous cycles in F1 females at all dose-levels.
In this study DBP appeared to be a reproductive toxicant in rats exposed both as adults and during development. The effects on the 2nd generation were greater than on the first generation. The lowest dose-level in this study, 0.1% in the diet (52 mg/kg bw/day for males; 80 mg/kg bw/day for females) is a LOAEL for embryotoxicity. The NOAEL for maternal toxicity is 0.5% in the diet (385 mg/kg bw/day)(NTP, 1995; Wine *et al.*, 1997).

In a fertility study female Wistar rats were exposed for 3 months before mating followed by a 7-day mating period to 0, 120 or 600 mg DBP/kg bw/day. Pregnant females were killed on day 21 of pregnancy. The study was not performed according to any guideline or under GLP conditions and the information given was limited. No maternal or embryotoxicity was seen in this study. 600 mg/kg bw/day is a NOAEL for embryotoxicity and maternal toxicity in this limited study (Nikoronow *et al.*, 1973).

In fertility studies in Charles River COBS CD rats, performed under GLP conditions, either male or female rats were exposed beginning 60 and 14 days, respectively, prior to mating, during mating, gestation and lactation. In the study in which females only were exposed, F1 weanlings were selected from all groups and were given either control diets or the same diets as their mothers for a 7 week post-weaning period (IRDC, 1984).

In the male fertility study, no effect on survival, appearance, behaviour, body weights, hematology and fertility was observed. Organ weights. of treated males showed a statistically significantly increased absolute as well as relative liver and kidney weight at 500 mg/kg bw/day. Relative kidney weights were also significantly increased in males at 50 and 5 mg/kg bw/day, but these increases were less pronounced, without a dose-relationship. Histopathology of the kidneys did not reveal abnormalities. In addition, well-performed 3 month rat studies revealed only at doses ≥ 350 mg/kg bw/day increased kidney weights. Therefore the increased kidney weights at 50 and 5 mg/kg bw/day seen in this male fertility study are considered as biologically insignificant. Reproductive performance, parturition, neonatal viability, growth of newborn, organ weights and histopathology in weanlings did not reveal abnormalities. The NOAEL for male fertility and embryotoxicity in this study is 500 mg/kg bw/day, the highest dose tested (IRDC, 1984).

In the female fertility study, no effect on survival, appearance, behaviour, hematology or fertility of treated females was seen. Growth of females was reduced slightly pre-mating, during the entire gestation period and during lactation period at 500 mg/kg bw/day, statistically significant at week 7, 9 and 11. At 50 mg/kg bw/day, also reductions in weight gain during the entire gestation period were seen, but less pronounced. Organ weights of treated females showed a statistically significant increase at 500 mg/kg bw/day. Histopathology did not reveal abnormalities. Reproductive performance, parturition and neonatal viability did not reveal abnormalities. Pup weight at birth and growth of pups

during entire lactation period was lower at 500 mg/kg bw/day. Organ weights and histopathology of weanlings did not show abnormalities. During the 7 week post-weaning period also reduced body weights were seen both with and without continuing treatment at all dose-levels, sometimes reaching statistical significance, but without any dose-relationship. Organ weights after 7 week post-weaning period revealed slightly decreased testicular weights in weanlings fed 500 mg/kg bw/day. After the 7 week post-weaning period, histopathology revealed testicular lesions in 6/10 weanlings (2 with mild granuloma unilateral, 1 with severe unilateral degradation, 1 with moderate bilateral degeneration, 2 with a trace of bilateral degeneration) fed 500 mg/kg bw/day. In the group derived from mothers fed 500 mg/kg bw/day and given control diet for 7 weeks post-weaning, 2/9 weanlings showed testicular lesions (1 with a trace of unilateral degeneration, 1 with severe unilateral degeneration). The NOAEL in this study is 50 mg/kg bw/day based on maternal toxicity and embryotoxicity (IRDC, 1984).

Conclusion

Male fertility of mice did not appear to be affected up to the highest dose-level of 1.0% in the diet (equivalent to 1410 mg/kg bw/day) in a one-generation study while female fertility was clearly affected at this dose-level. At 1.0% in the diet also embryotoxic effects were observed. The NOAEL in this study in mice is 0.3% in the diet equivalent to 420 mg/kg bw/day based on effects on maternal fertility and embryotoxicity.

Concerning the available reproduction studies in rats a NOAEL of 50 mg/kg bw/day can be established based on embryotoxicity in a one-generation reproduction study with exposure of females only. The same study protocol with exposure of male animals only, gave a NOAEL of 500 mg/kg bw/day.

However in a two-generation reproduction study in rats with a continuous breeding protocol and with exposure of both male and female animals the lowest dose-level of 0.1 % in the diet (52 mg/kg bw/day for males and 80 mg/kg bw/day for females) appeared to be a LOAEL based on embryotoxic effects (NTP, 1995; Wine *et al.*, 1997). It has to be noted (Foster, 1997) that the LOAEL of 52 mg/kg bw/day (0.1% in the diet) was derived from a more extensive study with improved sensitive endpoints (such as sperm parameters, estrous cycle characterization and detailed testicular histopathology)) compared to the study with the NOAEL of 50 mg/kg b.w. According to this author, the protocol of the continuous breeding study was supposed to identify adequately compounds with endocrine activity.

In conclusion, effects on pup weight and number of live pups per litter were seen in the absence of maternal toxicity at the lowest dose-level of 52 mg/kg bw/day in a 2-generation reproduction study in rats with a continuous breeding protocol. Other available reproduction studies in rats showed effects on fertility and embryotoxic effects at oral doses ≥250 mg/kg b.w.

Developmental studies

Developmental studies in mice and rats have been performed. None of these studies was performed according to any guideline and no data on GLP conditions were available.

# ANNEX 2

## Extracts from CSTEE opinions on the results of the Risk Assessment of DBP

### Opinion expressed on 24-4-1998 (issued from opinion on phthalate migration from soft PVC toys and child-care articles).

**Part on effects assessment**

In contrast to other phthalates like DINP and DEHP, DBP is not added intentionally to soft PVC toys and child-care articles. However, DBP can be present in these toys as by-product/impurity (in trace amounts), due to the use of technical phthalate mixtures in the production process.

Similar to other phthalate esters application of DBP to laboratory animals results in peroxisome proliferation, increased liver weight, liver tumours in mice, atrophic testes, impaired fertility and embryonal development. The monoester is seen as the active toxic metabolite. There is no indication that DBP or the metabolites are genotoxic (WHO, 1997).

In the gut and liver DBP is hydrolysed to phthalic acid, n-butanol and the monobutylphthalate (White *et al.*, 1983). The monoester is partially glucuronidised and excreted via the urinary tract. In rats the glucuronidation is 3-4 times lower than in hamsters, whereas glucuronidase activity in testes is higher, possibly explaining the higher sensitivity of rat testes to DBP toxicity.

Jobling *et al.* (1995) reported that DBP could reduce the binding of 17β-estradiol to the oestrogen receptor and stimulate transcriptional activity. To evaluate the relevance of these findings for the intact animal, the reproductive toxicity of DBP was studied using the NTP's Reproductive Assessment by Continuous Breeding (RACB) protocol (Wine *et al.*, 1997). Levels of 0.1, 0.5 and 1.0% DBP in the diet were selected, which yielded average daily DBP doses of 52, 256 and 509 mg/kg bw/day for males and 80, 385 and 794 mg/kg bw/day for females. If the findings of Jobling *et al* (1995) were correct one would see greater reproductive effects of DBP in second generation animals, because under the RACB protocol F0 rats are exposed only as adults, whereas F1 animals are born to mothers that are treated during maturation to sexual maturity and through mating. DBP consumption by F0 rats reduced the total number of live pups per litter in all treated groups by 8-17% and live pup weights in the 0.5% and 1.0% dose groups by more than 13%. In the pups reduced number of live pups, body weights, and in the F0 animals increased kidney and liver weights have been observed at the 1% dose (509 mg/kg bw/day for males and 794 mg/kg bw/day for females). In the F1 mating trial, indices of mating, pregnancy, and fertility in the 1% dose group were decreased, concomitant with a 13% decrease in the dams body weight. Necropsy resulted in decreased epididymal sperm contents and testicular spermatid head counts. No such effects have been seen at the 0.5% dose (256 mg/kg bw/day in males, 385 mg/kg bw/day in females). The F2 pup weights were 6-8% lower in all dose groups. This study shows that the reproductive/developmental effects of DBP in the second generation were greater than on the first generation. For reproduction the NOAEL was 256 mg/kg bw/day in males and 385 mg/kg bw/day in females. In the second generation the LOAEL for reduced F2 pup weights was 52 mg/kg bw/day for males and 80 mg/kg bw/day for females.

The critical effect used for assignment of a NOAEL value for DBP is reduced F2 pup weights observed in a 2-generation reproductive study with rats (Wine *et al*, 1997). This study did not identify

a NOAEL value, the LOAEL value for the critical effect was 52 mg/kg bw/day. Because the LOAEL value is used for calculating the TDI an additional uncertainty factor of 5 is used. A TDI of 100 µg/kg bw/day is assigned to DBP.

**Opinion expressed on 24-4-2001**

**Part on effects assessment**

DBP is rapidly absorbed and excreted after oral exposure. In rats dermal absorption appears to be in the range of 10% of the oral absorption. From an *in vitro* study it seems that human dermal absorption is only 2.5% of that in rat. It is the opinion of the CSTEE that this is not sufficiently discussed in the risk assessment. The CSTEE emphasises the need for more data to quantitate the probable difference in dermal absorption between humans and experimental animals. No data on absorption following inhalation exposure are available. The major part of absorbed DBP is hydrolysed to the monoester metabolite and further glucuronidated, or is subjected to oxidation leading to hydroxy and/or keto metabolites.

It is generally assumed that free MBP is the active, toxic metabolite of DBP. For the evaluation of the sensitivity of humans to DBP toxicity it is important to have information on the level of free MBP in the target tissues in humans compared to that in test animals. In the study by Blount *et al.* (2000), it was found that human urinary MBP was predominately conjugated as the glucuronide form. However, in 5% of the tested urinary samples the authors found a substantially higher concentration of unconjugated MBP.

The CSTEE agrees that the acute toxicity of DBP is low, but emphasises that the acute toxicity following inhalation exposure is difficult to assess. The CSTEE agrees that DBP is not a skin or eye irritant. However, irritation of nasal mucous membranes have been reported for mice after exposure by inhalation for 2 h to 0.25 mg/L and cats after receiving .1 mg/L for 5.5 h. Repeated exposure of rats by inhalation to concentrations = 1.18 mg/m3 induced adverse histopathological effects in the nasal cavity and larynx. These local, irritating effects in the upper respiratory tract give cause for some concern, however, due to an obvious lack of inflammation the CSTEE agrees with the conclusion of the RAR that DBP should not be classified as a respiratory irritant.

DBP has not been shown to be a skin sensitiser in well-accepted animal tests. Allergic dermatitis in humans has been reported in several studies using antiperspirants, nail polish and after contact with plastics containing DBP (watchbands, etc).
The CSTEE is aware of studies that indicate that dermal exposure to DBP may enhance the sensitisation potential of other skin sensitisers, possibly by acting as an adjuvant. DBP, in a dose-dependent fashion augmented the ability of topically applied FITC to stimulate proliferative responses in mice by draining lymph node cells, a correlate of skin sensitising potential. DBP also increased the frequency of lymph node dendritic cells bearing antigen (FITC positive DC), and increased the median amount of FITC antigen per dendritic cell. *In vitro* skin absorption studies also indicated that DBP increased the dermal absorption of FITC marginally. Exposure of mice to DBP alone did not give rise to any of the mentioned effects. The CSTEE finds that the possible adjuvant effects of DBP on other skin sensitisers should be commented upon in the RAR, and more studies on the adjuvant effects of DBP both on skin and respiratory sensitisers are warranted.

Several repeated-dose oral studies have been conducted in mice and rats. The quality of the reported studies varies, and some are not suitable for risk assessment. The key studies appear to be the NTP (1995) mice and rat studies and the rat study by Schilling *et al.* (1992). The NOAEL of 152 mg/kg/day from the Schilling study has been used in the risk assessment. In these studies changes in several

haematological parameters have been used as the critical effect used to determine the NOAEL. The Schilling study is not available in the open literature (confidential study by BAYER). A more detailed description of this study in the RAR is needed in order to asses the quality of this study. It is not clear how many doses were used, from the text it could be only two doses. The NTP studies are well performed. The CSTEE recommends that the NOAEL of 177 mg/kg bw/day from the NTP rat study (based on statistical significantly decreased haemoglobin values and erythrocyte counts together with increased numbers of blood platelets) should be used as basis for the risk assessment of systemic effects. The NTP studies, as well as several other oral studies of varying lengths, clearly show that the testis is a target tissue for DBP toxicity. In animals there are clear species differences to DBP-induced testicular toxicity. The CSTEE agrees with the RAR that the NOAEL for peroxisome proliferation is not used in the risk assessment.

An overall evaluation of bacterial mutagenicity tests shows that DBP is not a bacterial mutagen. Furthermore, no cytogenetic effects have been noted in various *in vitro* cell systems. In addition, DBP was negative in one cell transformation test. Two *in-vivo* micronucleus tests are negative. Unfortunately, most of the reported *in vitro* mutagenicity studies have not been reported in sufficient detail to allow an evaluation regarding their quality. Regarding gene mutation in mammalian cells the results are somewhat contradictory. The CSTEE has evaluated a new mouse lymphoma test (Barber *et al.*, 2000) that shows a positive effect in the presence, but not in the absence of a metabolism system (S9 mix). The fact that one study showed negative effects without S9 mix (not tested with S9 mix), but two were positive in the presence of S9 mix, indicates that DBP may cause gene mutation in cells in the presence of a metabolic activation system. However, recognising the relatively high rate of false positives in the mouse lymphoma assay and the overall negative responses in all other tests, the CSTEE agrees that DBP cannot be characterised as being genotoxic.

DBP has not been tested for carcinogenicity in experimental systems, nor are there any human data available. DBP has been documented to enhance peroxisome proliferation in rats and mice. Many peroxisome proliferators have been shown to cause liver tumours when given at high doses and for long periods in mice and rats. Based on the observations that humans are non-responsive to peroxisome proliferation, the CSTEE agrees that the peroxisome proliferative effect of DBP in rats and mice is of no relevance to humans. The male reproductive system is considered to be a main target of DBP toxicity. A recent study by Mylchreest *et al.* (2000) established NOAEL (50 mg/kg bw/day) and LOAEL (100 mg/kg bw/day) values for toxicity of DBP on male reproductive development in the F1 generation. The CSTEE considers this study to be very relevant for the risk assessment of reproductive toxic effects of DBP. This study should be used together with the 2-generation rat study that established a LOAEL of 52 mg/kg bw/day for embryotoxicity in the F2-generation, in the evaluation of the risk of reproductive toxicity.