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

Butylbenzylphthalate (BBP) for use in food contact materials

Question N° EFSA-Q-2003-190

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 butylbenzylphthalate (BBP) for use in the manufacture of food contact materials.

Previously, a temporary Tolerable Daily Intake (t-TDI) of 0.1 mg/kg bw was set by the Scientific Committee for Food (SCF) based on the end-point 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 BBP relate to reproduction. From the different studies available, the critical observations were as follows.

Decreases in epididymal spermatozoal concentrations have been reported at dose levels of 200 mg/kg bw/day and 2200 mg/kg bw/day, with a No Observed Adverse Effect Level (NOAEL) of 20 mg/kg bw/day and a Lowest Observed Adverse Effect Level (LOAEL) of 200 mg/kg bw/day. However, accompanying histopathological effects on the testes and adverse impact on fertility were only seen at 2200 mg/kg bw/day.
A NOAEL of 20 mg/kg bw/day and a LOAEL of 100 mg/kg bw/day were reported from a two-generation study, based on increased serum follicle stimulating hormone (FSH) concentrations in the F_0 parental males. Furthermore, in the same study all other examined end-points had a NOAEL of 100 mg/kg bw/day.
In another developmental toxicity study in the rat using a multiple dose design, foetotoxicity effects were observed. The overall benchmark dose, based on a 1% increase in abnormal testis location, was assessed at 95 mg/kg bw/day.
A multi-generation study including the F2 generation had an overall NOAEL of 50 mg/kg bw/day, based on the reduction in anogenital distance in F1 and F2 offspring at 250 mg/kg bw/day.

Based on the current literature on BBP testicular toxicity, the Panel allocated a Tolerable Daily Intake (TDI) of 0.5 mg/kg bw, derived from a NOAEL of 50 mg/kg bw/day found in a multi-generation study and making use of an uncertainty factor of 100.

The limited available data on BBP concentration in foods and diets in UK and Denmark were used to provide an estimation of the dietary exposure. In the UK, mean and high (97.5 percentile) intakes of BBP from dietary sources were estimated to be respectively 0.008 and 0.020 mg/person/day in the adult population (equivalent to 0.1 and 0.3 µg/kg bw/day).

In a Danish study, estimated mean exposure ranged from 0.02 to 0.03 mg/day, i.e. 0.3 to 0.4 µg/kg bw/day considering a 70 kg adult. Based on the highest concentration of BBP determined, exposure at high percentiles was estimated as 0.32 mg/day equivalent to 4.5 µg/kg bw/day.

http://www.efsa.eu.int/science/afc/catindex_en.html

In another Danish study, the main dietary sources of exposure were estimated to be root crops (30%) and leaf crops (60%). The total daily oral intake at the regional level (Denmark) can be estimated to 1 µg/kg bw/day in the adults, 5.9 µg/kg bw/day in children aged 1 to 6 years and 2.4 µg/kg bw/day in children aged 7 to 14 years.
Based on the detection limit, intake from infant formulae was estimated 1.6 µg/kg bw/day in infants of less than 6 months and 0.7 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 0.9 µg/kg bw/day.

The Panel noted that the dietary exposure to BBP (derived from packaging and other sources) may contribute up to about 1% of the TDI value.

KEY WORDS :

Butylbenzylphthalate, BBP, food contact materials, CAS n°85-68-7.

BACKGROUND

BBP may be present in food, either due to migration from food contact materials containing BBP or due to its widespread presence as an environmental contaminant which can be found in air, water, soil and food. BBP was evaluated by the Scientific Committee for Food (SCF) in 1988 and 1993 when a temporary Tolerable Daily Intake (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 BBP for use in food contact materials.

TERMS OF REFERENCE

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

ASSESSMENT

1. Chemistry

Identification of the substance

CAS No.:85-68-7

EINECS No.:201-622-7

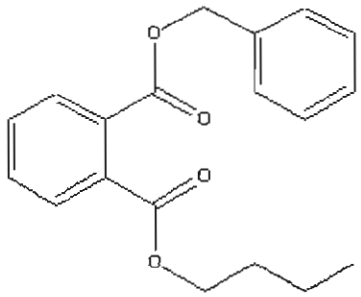
IUPAC name:benzyl butyl phthalate

FCM Ref N°74560

Synonyms: 1,2-benzenedicarboxylic acid, butyl phenylmethyl ester; benzyl-n-butyl phthalate; phthalic acid, butyl benzyl ester.

Molecular formula: $C_{19}H_{20}O_4$

Structural formula:



Molecular weight:312.35

Purity/impurities

Degree of purity:>98.5 % (w/w)

Identity and percentage (w/w) of impurities:

< 1.0 % di-benzyl phthalate(CAS No. 523-31-9)

< 0.5 % benzyl benzoate(CAS No. 120-51-4)

< 0.5 % di-butyl phthalate(CAS No. 84-74-2)

<2 ppm α-chlorotoluene(CAS No. 100-44-7)

<2 ppm α-α-dichlorotoluene(CAS No. 98-87-3)

Physico-chemical properties

Physical state: oily liquid

Melting point: - 35 °C

Boiling point: 370 °C at 1010 hPa

Relative density: 1.120 g/cm^3 at 20 °C

Vapour pressure:	$8.2 \pm 3.1 \times 10^{-5}$ hPa at 25 °C	
Water solubility:	2.8 mg/l at 20 °C	
Partition coefficient	n-octanol/water:	log K_{ow} 4.84

2. Use

BBP is present in a large number of end products some of which are available for consumer use. The vast majority of BBP use, more than 90%, goes to plasticizing of PVC or other polymers. The BBP plasticized polymeric material has consumer and industrial uses such as flooring, sealants, and paints.

3. Exposure via food

A relatively small but significant use is in the food wrap or food packaging area. BBP has also been reported at low concentrations in baby equipment and children toys. However, in these products BBP probably occurs as by-product/impurity and has not been added intentionally (CSTEE, April, 1998).

No data on the levels of BBP 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 BBP in samples of foods or diets allow to assess overall dietary exposure, from packaging material and other sources.

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 (97.5 percentile) intakes of BBP from these sources were estimated to be respectively 0.008 and 0.020 mg/person/day in the adult population equivalent to 0.1 and 0.3 µg/kg bw/day for a 60 kg adult (MAFF, 1996).

In a Danish study (Petersen and Breindahl, 2000), BBP 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, 8 were above the limit of determination. The estimated mean concentration of BBP in the diet varied according to the values assigned to the 21 samples which were under the limit of determination. Estimated mean exposure therefore ranged from 0.02 to 0.03 mg/day, i.e. 0.3 to 0.4 µg/kg bw/day considering a 70 kg adult. Based on the highest concentration of BBP determined, exposure at high percentiles was estimated as 0.32 mg/day equivalent to 4.5 µg/kg bw/day.

A further Danish assessment of BBP 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 (30%) and leaf crops (60%). These high contributions of vegetables to oral BBP 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 µg/kg bw/day in the adults, 5.9 µg/kg bw/day in children aged 1 to 6 years and 2.4 µg/kg bw/day in children aged 7 to 14 years. These values do not differ in a large extent from the UK figures mentioned previously.

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. Based on the maximum measured concentration among 11 commercial products (0.01 mg /kg of wet weight), this would lead to an exposure of 1.6 µg/kg bw/day in the infant of less than 6 months and 0.7 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 0.005 mg BBP/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 0.9 µg/kg bw/day.
In this study, total oral exposure was also assessed; it was estimated to be 0.97, 4.1, 5.9 and 2.4 µg/kg bw/day in adults, infants (6-12 months), children (1-6 years) and children (7-14 years), respectively. Total exposure from all sources including dermal and inhalation exposure was very similar: 1.0, 4.2, 6.0, and 2.5 µg/kg bw/day respectively, due to a very low estimated exposure from these sources via these two routes.

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 BBP Risk Assessment Report (RAR), prepared for the European Union Existing Substances Regulation, 793/93, 2001 (Annex 1), and the comments of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) on this RAR (Annex 2), in order to determine the most significant toxicological end-point for risk assessment. Based on this information, the Panel focused on the most sensitive toxicological end-points for the evaluation of BBP, taken from the reproduction/developmental toxicity studies with this substance.

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

> "Reviewing the evaluations on the phthalate esters and comparing the data with the data on di(2-ethylhexyl)phthalate, the phthalate ester studied most extensively, the Committee decided to use a safety factor of 1000 instead of 100 for calculating the TDI for butylbenzylphthalate. This means that a temporary TDI of 0.1 mg/kg bw was established and that a definite TDI can be established when an adequate teratogenicity study and a no-effect level for peroxisomal proliferation, based on liver weight, peroxisomal associated enzyme activities and ultra-microscopy of the liver are available."

Studies considered by the AFC Panel

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

A 26-week oral study in F344 rats resulted in decreased testicular weights, atrophy of seminiferous tubules, a near total absence of mature sperm production and marked hypospermia in the epididymis at dose level of 2.5% in the diet (1417 mg/kg bw/day) (Hazelton, 1985).

In conjunction with the NTP carcinogenicity study on BBP (NTP, 1997) a 10-week modified mating study in rats was also performed. Decreases in epididymal spermatozoal concentrations have been reported at dose levels of 200 mg/kg bw/day and 2200 mg/kg bw/day, with a NOEL of 20 mg/kg bw/day and a LOAEL of 200 mg/kg bw/day. However, accompanying histopathological effects on the testes and adverse impact on fertility was only seen at 2200 mg/kg bw/day (NTP, 1997a).

A NOAEL of 20 mg/kg bw/day was reported from a two-generation study, based on increased serum follicle stimulating hormone (FSH) concentrations in the F_0 parental males (Nagao *et al.*, 2000). The corresponding LOAEL was 100 mg/kg bw/day. In the F_1 generation, the NOAEL was 100 mg/kg bw/day. Furthermore, in the same study all other examined end-points had a NOAEL of 100 mg/kg bw/day.

In another developmental toxicity study in the rat using a multiple dose design (Piersma *et al,* 2000), BBP induced increased liver and kidney weights in dams, accompanied by liver enzyme increases in maternal serum. Observed foetotoxicity included increased resorptions, reduced foetal weights, increased incidence of skeletal anomalies, and reduced foetal testis weights in the presence of an increased incidence of retarded testicular descent. As embryotoxicity was found at lower dosages compared to observed maternal toxicity, BBP appeared to be a specifically embryotoxic compound. The overall benchmark dose was assessed at 95 mg/kg bw/day, based on a 1% increase in abnormal testis location.

In a multi-generation study (Tyl et al, 2001, 2004), BBP was administered in the diet at 0, 750 (50), 3750 (250), and 11,250 mg/kg (750 mg/kg bw/day) ad libitum. Adult F0 systemic toxicity and adult F1 systemic and reproductive toxicity were present at 11,250 mg/kg (750 mg/kg bw/day). At 11,250 mg/kg, there were reduced F1 and F2 male anogenital distance (AGD) and body weights/litter during lactation, delayed acquisition of puberty in F1 males and females, retention of nipples and areolae in F1 and F2 males, and male reproductive system malformations. At 3750 mg/kg (250 mg/kg bw/day), only reduced F1 and F2 offspring male AGD was present. There were no effects on parents or offspring at 750 mg/kg (50 mg/kg bw/day). The F1 parental systemic and reproductive toxicity NOAEL was 3750 mg/kg. The offspring toxicity NOAEL was 750 mg/kg (50 mg/kg bw/day), based on the presence of reduced AGD in F1 and F2 males at birth at 3750 mg/kg, but no effects were observed on reproductive development, structures, or functions.

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 BBP. Previous reviews have identified as pivotal several rat reproduction studies conducted in the last decade, which gave NOAELs or LOAELs in the region of 20-100 mg/kg bw/day, with the critical effect being on male reproductive development.

Based on the current literature on BBP testicular toxicity and on the presence of reduced AGD in F1 and F2 males at birth at 250 mg/kg bw/day, (NOAEL 50 mg/kg bw/day) in the Tyl *et al.* study (2001, 2004), the Panel allocated a Tolerable Daily Intake (TDI) of 0.5 mg/kg bw, derived from a NOAEL of 50 mg/kg bw/day and making use of an uncertainty factor of 100.

The limited available data on BBP concentration in foods and diets in UK (1993) and Denmark (2003) were used to provide an estimation of the dietary exposure. In the UK, mean and high (97.5 percentile) intakes of BBP from dietary sources were estimated to be respectively 0.008 and 0.020 mg/person/day in the adult population (equivalent to 0.1 and 0.3 µg/kg bw/day).
In a Danish study (Petersen and Breindahl, 2000), BBP estimated mean exposure ranged from 0.02 to 0.03 mg/day, i.e. 0.3 to 0.4 µg/kg bw/day considering a 70kg adult. Based on the highest concentration of BBP determined, exposure at high percentiles was estimated as 0.32 mg/day equivalent to 4.5 µg/kg bw/day.
In another Danish study (Müller *et al*, 2003), the main dietary sources of exposure were estimated to be root crops (30%) and leaf crops (60%). The total daily oral intake at the regional level (Denmark) can be estimated to 1 µg/kg bw/day in the adults, 5.9 µg/kg bw/day in children aged 1 to 6 years and 2.4 µg/kg bw/day in children aged 7 to 14 years.
Based on the detection limit, intake from infant formulae was estimated to be 1.6 µg/kg bw/day in infants of less than 6 months and 0.7 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 0.9 µg/kg bw/day.

The Panel notes that the dietary exposure to BBP (derived from packaging and other sources) may contribute up to about 1% of the TDI value.

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ANNEX 1

Extract from the Risk Assessment Report on BBP

Consolidated Final Report (dated February 2002)

Mutagenicity and carcinogenicity

BBP showed no evidence of mutagenicity in *Salmonell typhimurium* or mouse lymphoma cells. BBP did not induce sister chromatid exchanges (SCE) or chromosomal aberrations (CA) in CHO hamster cells. BBP induced morphological transformation in Syrian hamster embryo cells, but not in the BALB/3T3 cell transformation system. It did not induce sex-linked recessive lethals *in Drosophila melanogaster* or dominant lethal mutations in mice. Positive results were obtained in a mouse bone marrow test for SCE , however the responses were week, and the SCE test was not repeated. For the induction of CA conflicting results were reported when different observations times were compared. No induction of micronucleus were reported in female rats after exposure to low doses of BBP (182.6 µg/kg bw/day during gestation and lactation). Based on the available data, and according to EU criteria, BBP should not be considered a mutagen.

Butyl benzyl phthalate was tested for carcinogenicity by oral administration in mice (NTP, 1982) and rats (NTP, 1982, 1997), including a dietary restriction study (NTP, 1997). No increase in the incidence of tumours were observed in mice. The results from the rat studies are summarised in Table 5. An increased incidence of mononuclear cell leukemias was reported in female rats at 12,000 ppm BBP. No significant increase was, however, found in two later studies with the same rat strain although a higher concentration was tested. An increased incidence of benign pancreatic tumours was seen in one conventional study in male rats, but not after dietary restriction. A marginally increased incidence of pancreatic adenomas occurred in female rats in a conventional study, but not after dietary restriction. Papillomas of the urinary bladder was marginally increased in female rats both in the conventionally study and after dietary restriction. Moreover, after dietary restriction and 32 months an increase in bladder carcinomas was found. The latter results are difficult to interpret as no historic controls are available. In one study in rats, butyl benzyl phthalate given prior to 7,12-dimethylbenz(a)antracene inhibited mammary carcinogenesis. Thus, BBP appears to be a borderline case between no classification for carcinogenicity and. Cat. 3. However, due to the lack of genotoxic effects no classification is proposed.

Toxicity for reproduction and development

Reproductive effects of BBP and its major metabolites MBuP and MBeP in rats following oral administration both by gavage or in the diet have been investigated in studies of different duration (from 4 days to 26 weeks, and in 2-generation studies). The main effects reported include a decrease in the relative weight of testis, damage to the testis, epididymis, prostate, seminal vesicle and to reduced epididymal sperm concentrations, and at high BBP concentrations reduced fertility, in addition to increases in relative liver and kidney weights.

As regards the effects on fertility or reproductive organs following administration of BBP to rats in the diet (Tyl et al., 2001; Agarwal et al., 1985; NTP, 1997; Hammond et al., 1987) or via gavage (Piersma et al., 1995; Piersma et al., 2000; Lake et al., 1978; Nagao et al., 2000), reduced mating and

fertility indices, decreases in testis weight, histopathological changes in testis, and hormonal changes have been reported. These effects have in the majority of the studies been reported at BBP doses equal to (Hammond et al., 1987, 4-week diet study) or higher than those which have induced other effects, such as variations in absolute and relative weights of the liver and kidney and histopathological changes such as atrophy in the liver and pycnotic nuclei, acinar atrophy and slight fibrosis in the pancreas. Exceptions includes, when BBP is administered by gavage, a 14 days and a 4 days study in Sprague-Dawley rats (Lake et al., 1978), and a 28 days study in Cpb-WU rats (Piersma et al., 2000). In the Lake 14 days study, minimal testicular atrophy was reported in one of three animals examined at 480 mg/kg bw/day (Lake et al., 1978). In the 4 day study atrophic changes in the testis in 3 of 6 animals at 800 mg/kg bw/day of BBP were reported. In the 28 days study by Piersma et al., 2000 a decrease in testosterone level was reported from 450 mg/kg bw/day. Exceptions include, when BBP is administered in the diet, a 10 week fertility study (NTP, 1997). In this study a dose-related decrease in epididymal spermatozoa concentration compared to control animals was reported from 200 mg/kg bw/day (2800 ppm) ($p \leq 0.05$) of BBP and the NOAEL from this study was 20 mg/kg bw/day of BBP. When taking into account days of recovery in males in the 10 week NTP study (days from positive sperm plug to necropsy) in a covariate analysis of variance, on the epididymal spermatozoa concentration, the decrease in epididymal spermatozoa concentration at 2800 ppm was not statistically significant at the 5 % level compared to control animals ($p = 0.07$). However, the dose-dependent decrease in epididymal spermatozoa concentration was still evident. In the parallel 26-week oral toxicity study in rats where BBP was administered in the diet (NTP, 1997) the control value for epididymal spermatozoa concentration may not be valid due to a reported possible inadequate mincing of the cauda epididymis tissue from control animals. The NOAEL for reduced epididymal spermatozoa concentration and fertility in the 26 week study was 550 mg/kg bw/day. In a new 2-generation study (Tyl et al., 2001) significantly reduced mating and fertility indices were reported in F1 parents to make F2 offspring at 750 mg/kg bw/day. In the same study a significantly reduced relative and absolute paired ovaries and uterus weight was reported in F0 females. In adult F1 males a significant increase in reproductive tract malformations were reported (53.33 % compared to 0 % in controls). No increases in reproductive tract malformations were reported in females. Systemic toxicity reported at 750 mg/kg bw/day was limited to organ weight changes (liver, kidney) in males and females and histopathological lesions graded as minimal in females. The NOAEL for fertility was 250 mg/kg bw/day from this study. In another recent two-generation study (Nagao et al., 2000), increased serum FSH in F0 males was reported from 100 mg/kg bw/day, and at 500 mg/kg bw/day a decreased testosterone level. In F_1 males (18 weeks old) a decrease in testis, epididymis and ventral prostate weight, a decrease in testosterone and LH levels, and athrophy of the seminiferous tubules with decreased number of germ cells, and a decreased number of sperm in the epididymis were reported, accompanied with reduced body weight and an increased relative liver and kidney weight. No effect on fertility was reported in this study at any dose levels (20, 100 or 500 mg/kg bw/day). From the Nagao (2000) study, no NOAEL value could be derived for effects on fertility or the reproductive organs in parent animals. The NOAEL value for developmental effects was 20 mg/kg bw/day based on reduced body weight in male and female F1 offspring from 100 mg/kg bw/day.

In the risk characterisation, a NOAEL value OF 20 mg/kg bw/day for effects on the reproductive organs could be used. This NOAEL value is based on a dose-dependent decrease in epididymal spermatozoa concentration from 200 mg/kg bw/day (NTP, 1997).

ANNEX 2

Extracts from CSTEE opinions on the results of the Risk Assessment of BBP

Opinion expressed on 24-4-1998

Part on effects assessment

A 26-week oral study in F344 rats resulted in decreased testicular weights, atrophy of seminiferous tubules, a near total absence of mature sperm production and marked hypospermia in the epididymes at dose level of 2.5% in the diet (1417 mg/kg b/w./day). Relative liver weight was increased at the 0.83% level (470 mg/kg bw/day), but not at the 0.28% level (159 mg/kg bw/day) (Hazelton, 1985).

In an oral 90-day study in rats at dose levels of 151 to 1069 mg/kg bw/day, increased liver weights were seen at all dose levels in females and at the mid and high dose in males. The increased liver weights in females were only slight at the two lower doses. Cecal weights were also increased at all dose levels in females, but not in males. Histopathological lesions of the pancreas were observed in males at the mid and high doses. The liver of high dose males had small areas of necrosis. The LOAEL of increased liver and cecal weights in females was 171 mg/kg bw/day. The NOAEL for increased liver weight in males was 151 mg/kg bw/day, the NOAEL for pancreatic effects was 381 mg/kg bw/day (Hammond et al., 1987).

Biochemical evidence of liver peroxisomal proliferation in male F344 rats was determined at all dose levels in males and was apparent from a dietary level of 0.6% (approx. 300 mg/kg bw/day). Moderate electron microscopical evidence of liver peroxisomal proliferation was observed at the 2.5% dietary level (approx. 1250 mg/kg bw/day, this dose was the only one examined) (BIBRA, 1985). Also, a recent combined long-term and carcinogenicity assay identified a dose level of 300 mg/kg bw/day as the lowest effect level for peroxisome proliferation (NTP, 1997).

In a two-year bioassay with F344 rats, females at the high dose (1.2% dietary level, 1200 mg/kg bw/day) had increased incidence of mononuclear cell leukaemia (18/50 vs. 7/49 in control and also at the 0.6% dietary level). There were no increased incidence of tumours in males, but they had to be killed at week 29-30 due to internal haemorrhaging (NTP, 1982). No increased tumour incidences were observed in B6C3F1 mice fed for 103 weeks with 0, 0.6 (840 mg/kg bw/day) or 1.2% dietary levels (1680 mg/kg bw/day) of BBP (NTP, 1982). The increased incidence of mononuclear cell leukaemia in rats (NTP, 1982) was not seen in a new 2-year dietary study in F344/N rats (NTP, 1997). In this study, a statistically increased incidence of pancreatic neoplasms was found in males.

BBP is not genotoxic in a number of *in-vitro* tests or in a dominant lethal study in mice (Ashby et al., 1994).

BBP induces embryotoxicity and teratogenic effects at a maternally toxic dose of 750 mg/kg bw/day at days 7-9 or 13-15 of gestation, this was not due to decreased maternal food intake (Ema et al., 1991 and 1993). BBP is also embryotoxic and teratogenic at maternally toxic dietary levels of 1.25-2.0% (NTP, 1990). An oral developmental reproduction study with BBP in Wistar rats is ongoing at the TNO, The Netherlands (Protocol P 470839).

BBP has shown oestrogen-like effects *in-vitro* (Jobling et al., 1995). Sharpe et al. (1995) have reported that exposure of rats to BBP in drinking water at a level of 1000 µg/l (corresponding to a dose of 50 µg/kg bw/day) during pregnancy and lactation results in a reduction in testis size in the male offspring on postnatal day 90. However, administration of 1000 µg BBP/L to pregnant AP rats during gestation and lactation was not found to lead to changes in the sexual development of pups of either gender (Ashby et al., 1997). An as yet unpublished drinking water study performed by the TNO (sponsored by ECPI/CMA) with a similar design as the Sharpe et al.-Study, has also not been able to duplicate the Sharpe et al.-findings.

BBP given s.c. for three consecutive days up to an amount of 5 mg, did not induce increased uterine weight in prepubertal mice (Coldham et al., 1997).

The most sensitive clear critical effect reported in the repeated dose experiments was increased liver and cecal weights in female rats at the lowest dose of 171 mg/kg bw/day after 90-day feeding. No effects were found in males at 151 mg/kg bw/day (Hammond et al., 1987).

In a review document (BIBRA, 1992) it is stated that degeneration of testes were seen at 480 mg/kg bw/day/d, but the investigation itself was confidential. The study by Sharpe et al. (1995) have reported testicular toxic effects in the offspring at a very low dose (50 µg/kg bw/day/d), but Ashby et al. and ECPI/CMA (unpublished) failed to confirm these findings. It thus seems unwarranted to base a NOAEL on the findings of Sharpe et al. (1995).

Conclusion. The critical effect used for assignment of a NOAEL value for BBP are increased liver weight observed in an oral 90-day rat study (Hammond et al., 1987). This study did not identify a NOAEL value in female rats, the LOAEL value for the critical effect is 171 mg/kg bw/day. Because the LOAEL value is used and there was only a slight increase in liver weights, an additional uncertainty factor of 2 has to be incorporated.

Opinion expressed on 27-11-1998

Part on effects assessment

In conjunction with the NTP carcinogenicity study on BBP (NTP, 1997) a 10-week modified mating study in rats was also performed. At a dose of 200 mg BBP/kg bw/day (2800 ppm in feed) to a group of rats, the epididymal spermatozoal concentration was significantly less than the controls. The NOAEL for these effects was 20 mg/kg bw/day. For its reassessment the CSTEE will apply this value instead of the LOAEL of 171 mg/kg bw/day (incorporating an additional uncertainty factor of 2), as was done in its opinion of 24 April 1998.

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

Di-isodecylphthalate (DIDP) for use in food contact materials

Question N° EFSA-Q-2003-195

Adopted on 30 July 2005

SUMMARY

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

There are two different di "isodecyl" phthalate products with different CAS numbers (68515-49-1 and 26761-40-0). According to the European Council for Plasticisers and Intermediates (ECPI) these two products are prepared essentially from the same starting materials, through an identical olefin oligomerisation process and through similar oxo alcohol manufacturing and phthalate esterification processes.
The two phthalates are considered fully interchangeable within their whole range of the market end uses. Therefore, in this document they are considered together.

Previously, a group Tolerable Daily Intake (g-TDI) of 0.15 mg/kg bw (with di-isononylphthalate – (DINP)) 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 usual critical effects of phthalates relate to liver, testicular and reproduction toxicities. From the several studies available on DIDP, the critical observations were as follows:

There is no indication of effects on reproductive organs from histological observation in repeated dose toxicity studies.

In a recent two-generation study in rats, the F2 offspring survival was decreased. Based on this effect, a no observed adverse effect level (NOAEL) of 33 mg/kg bw/day could be established.

In a 13-week oral study in dogs, liver changes were seen at higher dose levels with a lowest observed adverse effect level (LOAEL) of 77 mg/kg bw/day and 88 mg/kg bw/day for male and female dogs respectively. The Panel concluded that the NOAEL of 15 mg/kg bw/day from this study should be used in the risk assessment.

Based on the liver effects in dogs (a species considered as a non-sensitive species to peroxisome proliferation) with a NOAEL of 15 mg/kg bw/day, and on a decrease of F2 offspring survival with a NOAEL of 33 mg/kg bw/day, a lowest overall NOAEL of 15 mg/kg bw/day has been considered. Making use of this NOAEL and of an uncertainty factor of 100, a TDI of 0.15 mg/kg bw is derived.

http://www.efsa.eu.int/science/afc/catindex_en.html

The limited data available on DIDP concentration in foods and diets in UK (1996, 1998) and Denmark (2003) were used to provide an estimation of the dietary exposure. In the UK, potential exposure to DIDP from dietary sources was based on the method detection limit and estimated to be less than 0.17 μg/kg bw/day. For newborns (0-6 months) and for infants (>6 months), the potential exposure to DIDP derived from infant formulae consumption corresponded to 2.4 μg/kg bw/day and 1.8 μg/kg bw/day respectively.

A Danish DIDP total oral exposure was reported recently and was estimated to be 3 μg/kg bw/day for adults. Higher values for total oral exposure (210, 53 and 7 μg/kg bw/day) were reported for infants (6-12 months), children (1-6 years) and children (7-14 years), respectively. However the two highest values for young children, derived mainly from the contribution of the estimated oral exposure related to toys that is included in the above values. DIDP use in toys is provisionally banned in the EU since 1999. Furthermore, the computer modeling program (EUSES) which was used for these intake estimates is a conservative one and the obtained values are not representative of the possible exposure via food contact materials. However, the value of 7 μg/kg bw/day from this study has been taken as a worst case estimate of dietary exposure to DIDP.

The Panel noted that the above estimated exposure via the diet of around 7 μg/kg bw/day is well below the TDI. However, there are some indications that DIDP levels in food may be increasing in recent years, and so, more up-to-date estimations of exposure from the diet are desirable.

The Panel noted also that DIDP and DINP (phthalic acid, diester with primary saturated C8-C10 branched alcohols, C9 rich, CAS n° 28553-12-0 and 68515-48-0, PM/REF 75100) are mixtures that overlap chemically with each other and cannot analytically be distinguished clearly if present in a mixture. For this reason, it is proposed that for DINP and DIDP a group restriction is established for migration from food contact materials.

KEY WORDS :

1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich (CAS n° 68515-49-1); di-''isodecyl'' phthalate (CAS N° 26761-40-0); di-isodecylphthalate; REF No. 75105, food contact materials

BACKGROUND

DIDP may be present in food, either due to migration from food contact materials containing DIDP or due to its widespread presence as an environmental contaminant which can be found in air, water, soil and food. DIDP was evaluated by the Scientific Committee for Food (SCF) in 1999 when a group TDI (with DINP) for use in food contact materials was established based on the then most sensitive end-point of peroxisome proliferation in rodent liver (SCF, 1999). 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 DIDP for use in food contact materials.

TERMS OF REFERENCE

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

ASSESSMENT

1. Chemistry

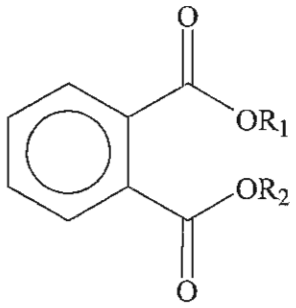
Identification of the substance

There are two different di "isodecyl" phthalate products with different CAS numbers (68515-49-1 and 26761-40-0). According to specific information from the European Council for Plasticisers and Intermediates (ECPI, 1996) these two products are prepared essentially from the same starting material, through an identical olefin oligomerisation process and through similar oxo alcohol manufacturing and phthalate esterification processes.
The two phthalates are considered fully interchangeable within their whole range of the market end uses.

CAS	68515-49-1	26761-40-0
EINECS-Nr	271-091-4	247-977-1
Substance name (IUPAC Name)	1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich	1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich (di-''isodecyl'' phthalate)
Molecular formula	$C_{28}H_{46}O_4$ (average)	
Molecular weight	446.68 (assuming the above average molecular formula)	

FCM Ref N°75105

Structural formula:



where R1 and R2 = C9-C11, C10 rich, linear and branched

The correct structures can only be estimated. Based on nonene (CAS 97593-01-6) isomer distribution analysis and 1H-NMR analysis of isodecyl alcohol, an estimation of key isomeric structures of isodecylalcohol, and hence of DIDP were provided by ECPI (1998).

Longest chain (estimates)	DIDP (CAS 68515-49-1 & CAS 26761-40-0)	Best estimated content (%)

C7	tri-methylheptanols	0-10
C8	di-methyloctanols	70-80
C9	methylnonanols	0-10
C10	n-decanol	0

Purity/impurities, additives

Phthalates are produced with a high degree of purity (> 99.5%), in terms of ester content. Trace impurities have been summarised from producers' data.

Diisodecyl ether and Isodecyl benzoate	0.02 - 0.1% w/w
Isodecyl alcohol	0.01 - 0.05% w/w
Water	max. 0.1% w/w

Physico-chemical properties

Property	Value
Melting point	-53 to -39°C (av. -45°C)
Boiling point	> 400°C
Density	0.966 at 20°C
Vapour pressure	5.1.10-5 Pa at 25°C
Water solubility	0.2 µg/l at 20°C
Log Kow	8.8

2. Use

1,2-benzenedicarboxylic acid, di-C9-C11-branched alkyl esters, C10-rich (CAS n° 68515-49-1, EINECS n° 271-091-4) / di-''isodecyl'' phthalate (CAS n° 26761-40-0, EINECS n° 247-977-1) is mainly used as plasticiser in PVC.

3. Exposure via food

No data on the levels of DIDP in food in the EU attributable to migration from food contact materials have been submitted by the industry.

In 1996, MAFF's Food Safety Directorate carried out a survey of the levels of DIDP and total phthalates in samples of composite fatty foods (MAFF 1996a). DIDP was not detected in the conditions of analysis (limit of detection = 0.01 mg/kg of food). Based on the detection limit, and

assuming a food intake per day of 1 kg for an adult of 60 kg, the potential daily exposure to DIDP from food would be < 0.17 μg/kg bw/day.
For newborns (0-6 months) and for infants (>6 months), the potential exposure to DIDP derived from infant formulae consumption, based on the detection limit, corresponds to 2.4 μg/kg bw/day and 1.8 μg/kg bw/day respectively (MAFF, 1996b and 1998).

A Danish DIDP total oral exposure was reported recently (Müller et al, 2003) and was estimated to be 3 μg/kg bw/day for adults. Higher values for total oral exposure (210, 53 and 7 μg/kg bw/day) were reported for infants (6-12 months), children (1-6 years) and children (7-14 years), respectively. But these two highest values for young children derive mainly from the contribution due to the estimated oral exposure related to toys that is included in the above values. DIDP use in toys is provisionally banned in the EU since 1999 (Commission Decision, 1999 and 2004). Furthermore, the computer modeling program (EUSES) which has been used for these intake estimates is a conservative one and the obtained values are not representative of the possible exposure via food contact materials. However, the value of 7 μg/kg bw/day from this study has been taken as a worst case estimate of dietary exposure to DIDP.

There are some indications that DIDP levels in food are increasing in recent years, and so, more updated estimations of exposure from the diet are desirable.

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 DIDP Risk Assessment Report (RAR), prepared for the European Union Existing Substances Regulation, 793/93, 2001 (Annex 1), and the comments of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) on this RAR (Annex 2), in order to determine the most significant toxicological end-point for risk assessment. Based on this information, the Panel focused on the most sensitive toxicological end-points for the evaluation of DIDP.

The SCF expressed its opinion on DIDP in December 1999 (SCF, 1999) based on the following statement in the safety data sheet:

> "The liver was identified as the target organ following oral administration of DIDP in rats and dogs. A dose-dependent increase in absolute liver weight was found. A NOAEL of 15 mg/kg bw/day could be derived from these studies, based on liver effects following repeated oral exposure.
> Making use of an uncertainty factor of 100 (10 for intraspecies and 10 for interspecies), a TDI of 0.15 mg/kg bw/day could be allocated to DIDP.
>
> DIDP and DINP (phthalic acid, diester with primary saturated C8-C10 branched alcohols, C9 rich and di-"isononyl"phthalate) being mixtures overlapping chemically each other it is decided to establish a group restriction of 9 mg/kg food, together with DINP (CAS n° 28553-12-0 and 68515-48-0, PM/REF. 75100)."

Studies considered by the AFC Panel

The following studies on genotoxicity, carcinogenicity, liver toxicity, reproduction and development toxicities considered by the Panel for the determination of a NOAEL which could be used as a basis for a TDI calculation are summarized below. Further details on these studies are given in Annex 1

(Risk Assessment Report) and Annex 2 (CSTEE opinion). Long-term studies on carcinogenicity for DIDP are not available.

DIDP is not mutagenic *in vitro* in bacterial mutation assays (with and without metabolic activation) and is negative in a mouse lymphoma assay. It is not clastogenic in a mouse micronucleus assay *in vivo*. This indicates that DIDP is a non-genotoxic agent.

Repeated-dose toxicity in rats produced peroxisome proliferation-related liver and thyroid effects accompanied by increases in liver weight (Hazelton Laboratories, 1968a, BASF, 1969 a and b). A NOAEL of 60 mg/kg bw/day was identified in the latter study based on increased relative liver weight in female rats. This study is not relevant for the determination of a TDI since the NOAEL was based on peroxisome proliferation.

In a 13-week oral study in dogs using dose levels of 0.05, 0.3 and 1% in the diet, liver changes (swollen and vacuolated hepatocytes and dose-related increases in liver weight increases) were seen at the two higher dose levels (Hazleton Laboratories, 1968b). A NOAEL of 15 mg/kg bw/day was identified by the study authors and by the RAR. The fact that dogs are considered to be non-responsive or refractory to peroxisome proliferation could indicate that minor liver damage found in this species occurred by a mechanism different to peroxisome proliferation. The Panel noted that the RAR and CSTEE opinions commented that the small number of animals in the study precluded statistical analysis and that the validity of this NOAEL may be in question. The Panel also noted that the NTP-CERHR Expert Panel concluded that it was not possible to derive a NOAEL for this study, and that a LOAEL of 77 mg/kg bw/day and 88 mg/kg bw/day for male and female dogs, respectively, should be considered (CSTEE opinion).

There is no indication of effects on reproductive organ from histological observation in repeated dose toxicity studies in rats (BASF, 1969b and 1995; Hazelton, 1968b) and in dogs (Hazelton, 1968b).

With respect to reproductive toxicity, decreases in survival indices were observed consistently in 2 two-generation studies with rats (Exxon Biomedical Sciences, 1997b; 2000). NOAELs of 38-44 and 52-114 mg/kg bw/day during pregnancy and lactation were identified for reproductive toxicity by the study authors. In the more recent two-generation study in rats (Exxon Biomedical Sciences, 2000), the F2 offspring survival was decreased. A NOAEL of 33 mg/kg bw/day (lowest estimated dose for 0.06% DIDP in the diet) could be derived for this effect.

The results of the one- and two-generation studies showed that DIDP did not affect fertility in rats.

With respect to developmental effects, skeletal variations (including rudimentary lumbar ribs and supernumerary cervical ribs) were observed in developmental studies in rats at 1,000 mg/kg bw/day concurrently with slight signs of maternal toxicity. A NOAEL of 500 mg/kg bw/day for maternal toxicity and a NOAEL of 100 mg/kg bw/day for developmental effects were established in the two-generation rat study (Exxon Biomedical Sciences, 1997b; 2000).

CONCLUSIONS

Based on all the available toxicological evidence, the Panel concluded that effects on liver, reproduction and development are the end-points on which to base the risk assessment. Previous reviews on phthalates have identified as pivotal several rat reproduction studies conducted in the last decade, which gave NOAELs or LOAELs in the region of 15-150 mg/kg bw/day.

There is no indication of effects on reproductive organs from histological observation in repeated dose toxicity studies (BASF, 1969b; Hazelton, 1968b).

In a recent two-generation study in rats (Exxon Biomedical Sciences, 2000), the F2 offspring survival was decreased. Based on this effect, a NOAEL of 33 mg/kg bw/day could be derived.

In a 13-week oral study in dogs, liver changes were seen at higher dose levels with a LOAEL of 77 mg/kg bw/day and 88 mg/kg bw/day for male and female dogs, respectively. The Panel concluded that the NOAEL of 15 mg/kg bw/day from this study should be used in the risk assessment.

Based on the liver effects in dogs (a species considered as a non-sensitive species to peroxisome proliferation), with a NOAEL of 15 mg/kg bw/day and on a decrease of F2 offspring survival with a NOAEL of 33 mg/kg bw/day, a lowest overall NOAEL of 15 mg/kg bw/day has been considered. Making use of this NOAEL and of an uncertainty factor of 100, a TDI of 0.15 mg/kg bw is derived.

The limited data available on DIDP concentration in foods and diets in UK (1996, 1998) and Denmark (2003) were used to provide an estimation of the dietary exposure. In the UK, potential exposure to DIDP from dietary sources was based on the method detection limit and estimated to be less than 0.17 µg/kg bw/day. For newborns (0-6 months) and for infants (>6 months), the potential exposure to DIDP derived from infant formulae consumption corresponds to 2.4 µg/kg bw/day and 1.8 µg/kg bw/day respectively,

A Danish DIDP total oral exposure was reported recently (Müller et al, 2003) and was estimated to be 3 µg/kg bw/day for adults. Higher values for total oral exposure (210, 53 and 7 µg/kg bw/day) were reported for infants (6-12 months), children (1-6 years) and children (7-14 years), respectively. However, the two highest values for young children derive mainly from the contribution of the estimated oral exposure related to toys that is included in the above values. DIDP use in toys is provisionally banned in the EU since 1999 (Commission Decision, 1999 and 2004). Furthermore, the computer modeling program (EUSES) which has been used for these intake estimates is a conservative one and the obtained values are not representative of the possible exposure via food contact materials. However, the value of 7 µg/kg bw/day from this study has been taken as a worst case estimate of dietary exposure to DIDP.

The Panel noted that the above estimated exposure via the diet of around 7 µg/kg bw/day is well below the TDI. However, there are some indications that DIDP levels in food have increased in recent years, and so, more up-to-date estimations of exposure from the diet are desirable.

The Panel noted also that DIDP and DINP (phthalic acid, diester with primary saturated C8-C10 branched alcohols, C9 rich, CAS n° 28553-12-0 and 68515-48-0, PM/REF 75100) are mixtures that overlap chemically with each other and cannot analytically be distinguished clearly if present in a mixture. For this reason, it is proposed that for DINP and DIDP a group restriction is established for migration from food contact materials.

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ANNEX 1

Extracts from the Risk Assessment Report on DIDP
Consolidated Final Report (dated May 2001)

Summary on mutagenicity and carcinogenicity

DIDP is not mutagenic *in vitro* in bacterial mutation assays (with and without metabolic activation) and is negative in a mouse lymphoma assay. It is not clastogenic in a mouse micronucleus assay *in vivo*. This indicates that DIDP is a non-genotoxic agent.

No carcinogenicity long-term study is available for DIDP but an increase in incidence of hepatocellular tumours in rats related to peroxisome proliferation might be anticipated, in regard with the increased incidence in tumour liver cells observed with DEHP and DINP in carcinogenicity studies. Thus, there is no concern in regard with carcinogenicity

Summary on repeated-dose toxicity

The repeated-dose studies are summarised in the following table.

Species	Treat-ment	Substance Purity/dose	Body Weight	Clinical Signs	Biochemistry/ Haematology	Effects on organs		NOAEL	Reference
						Macroscopy	Microscopy		
Young Rat Fisher 344	21 days in diet	DIDP 99.64% pure 0-0.3-1.2-2.5%	↓ body weight 2.5%	No change	↓ serum triglycerides and cholesterol (1.2 and 2.5% males) ↑ cyanide – insensitive palmitoyl – CoA oxidation 1.2%-2.5% (female and male) ↑ 11 and 12 – hydroxylation of lauric acid 0.3-1.2-2.5% (males) ↑ 12 hydroxylation of lauric acis 2.5% (females)	↑ liver weight from 0.3% (males) from 1.2% (females) Kidney weight ↓ 2.5% Slight ↓ absolute testicular weight 2.5%	↓ hepatocyte cytoplasmic basophilia 1.2 and 2.5% ↑ eosinophilia (2.5%) No testicular change	0.3% (304 mg/kg/d for males) (264 mg/kg/d for females)	BIBRA (1988)
Rat Fisher 344	28 days in diet	0.020-0.05-0.1-0.3-1%	No change	-	↑ cyanide insensitive palmitoyl CoA oxidation from 0.1%	No testicular atrophy	No testicular atrophy	0.05% (57 mg/kg/d)	Lake et al. (1991)
Rat Sprague Dawley	28 days in diet	Palatinol Z 5,000 and 10,000 ppm	Slight ↓ in males	No change	No change	↑ liver weight 5,000 and 10,000 ppm	No change	5,000 ppm (600 mg/kg/d for males) (1,100 mg/kg/d for females)	BASF (1988a)
Rat Sprague Dawley	90 days in diet	Palatinol Z 800-1,600-3,200 & 6,400 ppm	Slight ↓ in male	No change	No change	↑ liver weight (absolute) at 6,400 ppm in males dose-related increase of liver weight from 1,600 ppm in females	No change No change	3,200 ppm (200 mg/kg/d for males) 800 ppm (60 mg/kg/d for females)	BASF (1988b)
Rat	90 days in diet	DIDP-FDA Grade 0.05-0.3-1%	Slight ↓ all doses in males 1% in females	No change	No change	↑ Liver weight 1%	No change	0.3% (200 mg/kg/d)	Hazleton (1988)
Dog (Beagles)	90 days in diet	0.05 - 0.3 - 1%	Slight ↓ 1%	No change	No change	↑ Liver weight 0.3-1%	swollen and vacuolated hepatocytes from 0.3%	0.05% (15 mg/kg/d)	Hazleton (1988)

Summary on toxicity on reproduction and fertility

In 42-44 day year old (pubertal) or adult rats there is no indication of organ reproductive effects evidenced by histological observation in repeated dose toxicity studies and the two-generation study. In the two-generation study decrease in mean percent normal sperm was observed but of low incidence and only in P1 generation. In pups (F1, F2 and in the cross fostering satellite group) decrease in testes weight and cryptorchidism in F2 high-dose offspring were observed likely due to the low body weight since no histopathological damages were observed in adult testes. There were no changes in Reproductive Indices. From those assays no adverse effects on fertility may be anticipated.
In regard with reproductive toxicity DIDP is a developmental toxicant since decrease in survival indices was observed consistently in both two-generation studies (Exxon Biomedical Sciences, 1997b; 2000) leading to the NOAEL of 0.06% (Exxon Biomedical Sciences, 2000). The NOAEL of 0.06% (33 mg/kg bw/day DIDP) is taken into account in the risk characterisation. In regard with developmental effects, skeletal variations are observed in the developmental studies at 1,000 mg/kg bw/day concurrently with slight signs of maternal toxicity and lead to a NOAEL of 500 mg/kg bw/day. In the two-generation rat study (Exxon Biomedical Sciences, 1997b) body weight decrease was observed in offspring partly related to lactation at the highest dose of 0.8% and leads to a NOAEL of 0.4% (253 to 761 mg/kg bw/day seeing that received doses are widely dependent on the period considered). Those NOAELs are considered for risk characterisation. No effects were seen on fertility thus no classification according to the EU is needed. With regard to development decrease in survival indices mainly in F2 (day 1 and day 4) in the two-generation study as well as skeletal variations in developmental studies are not severe enough to justify a classification.

ANNEX 2

Extract from CSTEE opinion on the results of the Risk Assessment of DIDP

Opinion expressed at the 24th CSTEE plenary meeting, 12 june 2001.

Repeated dose toxicity

DIDP has been studied for repeated dose toxicity mainly in rats, but results from studies using other species such as rabbit, cat and dog have also been reported. The route of administration is primarily by ingestion. Only one inhalation study was located. In this 14-day study in rats at 0.5 mg/l (aerosol) only a local irritant effect, but no systemic toxicity was found. The RAR assumes a NOAEL (systemic toxicity) of 0.5 mg/l. No studies using dermal exposure were located.

The liver was identified as the target organ following oral administration of DIDP in the feed for 28 or 90 days in rats. The liver effects found are consistent with peroxisome proliferation. In one of the 28-day repeated dose study reported (BASF 1969a) in the RAR a NOAEL of 600 mg/kg bw/day is given. However, in the NTP-CERHR of 2000 it is reported that all doses tested resulted in an increased absolute and relative liver weight. Thus, it seems that a NOAEL of 600 mg/kg bw/day may not be correct. In a 21-day feeding study in rats a NOAEL for increase in absolute liver weights in females was identified to be 264 mg/kg bw/day. In a second 28-day study a NOAEL for increased absolute liver weight was 57 mg/kg bw/day in male rats. Thus, it appears that female rats are somewhat more susceptible to DIDP-induced liver toxicity than males. Two 90-day studies are reported in the RAR. In the first of these studies (BASF, 1969b) a NOAEL for increased absolute liver weight of 200 mg/kg bw/day is assumed for male rats and 60 mg/kg bw/day for female rats based on relative liver weights. It is not clear why in male rats the NOAEL is based on absolute liver weight and in females on relative liver weight, especially since relative liver weights were increased at all dose levels tested in male rats. If relative liver weights are used also in males a LOAEL of 55 mg/kg bw/day is derived. In the second 90-day study (Hazelton Laboratories, 1968a) a NOAEL of 200 mg/kg bw/day is assumed based on increased liver weight and a minimal increase in thyroid activity. In addition to the rat studies one study in dogs has been reported. A dose-dependent increase in absolute liver weight was found, but the small number of animals used precluded statistical analysis. The RAR has assumed a NOAEL of 15 mg/kg bw/day whereas the NTP-CERHR Expert Panel has concluded that is not possible to derive a NOAEL for this study. Thus, a LOAEL of 77 mg/kg bw/day and 88 mg/kg bw/day for male and female dogs, respectively, should be considered. No relevant human data are available.

Mutagenicity

DIDP has been tested for mutagenicity *in vitro* and *in vivo*. No mutagenicity was found in bacterial tests or in mammalian cells (mouse lymphoma test). In the mouse lymphoma study DIDP was incompletely soluble and formed oily droplets at all concentrations tested. However, cytotoxicity was noted indicating that a sufficiently high concentration of DIDP was achieved. In the mice bone marrow micronucleus test DIDP was administered by gavage and not ip, which is the preferred route of administration to detect cytogenetic effects. DIDP was negative. The CSTEE agrees with the conclusion that the limited data available indicate that DIDP is non-genotoxic.

Carcinogenicity

DIDP has not been tested for carcinogenicity in experimental animals nor are there any available human data. Being a peroxisome proliferator one would suspect it to cause liver tumours in rat and/or mice carcinogenicity studies, as observed with other phthalates (DEHP and DINP). Both positive and negative cell transformation assays have been reported. The fact that DIDP does not interact with DNA and that peroxisome proliferation is generally accepted not to be associated with liver cancer in humans, leads to the conclusion that DIDP does not cause a concern for human cancer.

Reproductive toxicity

With respect to developmental toxicity two studies in rats and one in mice were found. No adverse effects on dams or offspring were noted when mice were administered a high dose of DIDP on gestation days 6-13. In the first rat study (Waterman et al., 1999) rats were administered DIDP by gavage on gestation days 6-15 at 0, 100, 500, or 1000 mg/kg bw/day. The CSTEE agrees with a NOAEL of 500 mg/kg bw/day for the dams. Waterman assumed a NOAEL for developmental effects of 500 mg/kg (based on skeletal variations on a per litter base). The NTP-CERHR Expert Panel Report (2000) disagreed with a developmental NOAEL of 500 mg/kg bw/day and a statistical re-evaluation of the data showed that a NOAEL of 100 mg/kg bw/day was more appropriate based on the incidence of cervical and accessory 14^{th} ribs. The re-analysed data shows a statistical increase at 500 mg/kg bw/day of skeletal variation, rudimentary lumbar ribs and supernumerary cervical ribs. Based on these data the CSTEE supports a NOAEL of 100 mg/kg bw/day for development. In the second rat study (Hellwig et al., 1997), rats (7-10 per dose group) were administered DIDP by gavage at 0, 40, 200, and 1000 mg/kg bw/day on gestation days 6-15. In this study a NOAEL of 200 mg/kg bw/day for maternal toxicity was derived and a NOAEL of 200 mg/kg bw/day for developmental toxicity (based on significant skeletal variation in the foetus) is assumed in the RAR. The CERHR Expert Panel, however, based on an increased incidence of dilated renal pelvis and hydroureter leading to a statistically significant increase in the mean percent of foetuses affected per litter with variations at the 200 and 1000 mg/kg bw/day, concluded that a NOAEL of 40 mg/kg bw/day was relevant. Based on the fact that several studies indicate that the effects on renal pelvis may be transient and that no renal effects were noted in the two-generation study, the CSTEE agrees that a NOAEL of 200 mg/kg bw/day is indicated. Further, the CSTEE agrees that an overall evaluation of the two rat studies suggests a maternal NOAEL of 500 mg/kg bw/day. However, the CSTEE does not support a NOAEL of 500 mg/kg bw/day based on skeletal variation. Both rat studies indicate a NOAEL of 100-200 mg/kg bw/day, and applying a conservative approach a NOAEL of 100 mg/kg bw/day is proposed.

Reproductive toxic effects have also been observed in one- and two-generation studies in rats.

No NOAEL could be derived from the one-generation study. In the first two-generation study no NOAEL of 253 to 761 mg/kg bw/day is assumed for developmental effects by the RAR. However, when the same study is evaluated by CERHR it is concluded that no NOAEL could be derived and that a reproductive toxic LOAEL of 131-152 mg/kg bw/day and 162-379 mg/kg bw/day in F_0 and F_1 dams during gestation and lactation, respectively, was appropriate. In the follow up two-generation study using lower doses, CERHR state that a reproductive toxic NOAEL of 38-44 and 52-114 mg/kg bw/day during pregnancy and lactation was identified by the study authors. The RAR concludes that no reproductive toxic effects were found at any dose tested. However, a NOAEL of 33 mg/kg bw/day could be derived for offspring toxicity in the F_2 generation (lowest estimated dose for 0.06% DIDP in the diet). The results of the one- and two-generation studies show that DIDP does not affect fertility in rats.

As stated above and based on the results of the prenatal studies, the CSTEE supports a NOAEL of 500 mg/kg bw/day for maternal toxicity. However, the CSTEE does not agree with the RAR in using of 500 mg/kg bw/day as a NOAEL for developmental toxicity. The CSTEE prefers a NOAEL of 100 mg/kg based on the re-evaluation of study data. Based on the second two-generation study, where a decrease in survival indices in the F_2 generation was noted, the CSTEE supports a NOAEL of 33 mg/kg bw/day as suggested in the RAR. The acceptance of 33 m g/kg bw/d as a NOAEL is based on NOAELs of 38-44 during pregnancy and 52-114 mg/kg bw/day during lactation, with respect to pup survival and growth in the cross-fostering and switched-diet satellite studies. Also, the first two-generation study showed a decrease in the survival index. The CSTEE does not agree that a NOAEL of 253 to 761 mg/kg bw/day should be used for the body weight decrease and prefer 127-151 mg/kg bw/day for gestation and 166-377 mg/kg bw/gay for lactation, as concluded by CERHR for the first two-generation study. The CSTEE agrees that DIDP should be considered a developmental toxicant. The CSTEE also agrees that DIDP does not affect fertility at doses up to 928 mg/kg bw/day based on the two-generation study and on the repeated dose studies in rats at doses up to 2100 mg/kg bw.