

Dipentylphthalat
(CAS-Nr.: 131-18-0)

Di-(n-pentyl)phthalate (DnPP) is a linear phthalic acid ester of medium chain length. Certain products also contain the isopentyl isomer in major amounts and some of the studies described below are based on this mixture. Major toxicological differences are not assumed for the isopentyl-isomer (di-iso-pentylphthalate; CAS No.: 42925-80-4) nor for the closely related di-n-butylphthalate (DBP) and di-n-hexylphthalate. Where data gaps exist, study results from either compounds may be cross-read to DnPP and considered for the assessment of this substance.

Mutagenicity:

No data are available for DnPP itself or its isomers.

On the basis of the structural characteristics and in analogy to the very extensive data base relating to di-n-butylphthalate (DBP; BUA 1987/1993), with regard to this end point, there are no suspicions of DnPP having a genotoxic effect.

Carcinogenicity:

Long-term studies on DnPP are not available.

An increase in liver weights was determined within the framework of a prenatal toxicity study conducted with a mixture of 40% DnPP and DIPP (see below). DnPP is presumably a peroxisome proliferator in the rodent in the same way as DBP (BUA 1987/1993). This type of enzyme induction is associated with a general enlargement of the liver and - at least initially - increased DNA synthesis. In the rat and the mouse, this potentially represents a metabolism situation in which there is a disposition to develop hepatic tumours.

However, there is a great difference in the actual carcinogenicity of the individual peroxisome proliferators. The level of the effect threshold and the extent of liver enlargement, rather than the maximum peroxisome density and enzyme activity in the high-dose range, are prognostically meaningful. Various lipid-reducing pharmaceutical agents as well as the phthalic acid ester DBP have been examined for this in detail. The phthalic acid esters belong to the weak peroxisome proliferators which means that relatively high doses are required to trigger this effect and carcinogenicity does not show up unless at a low rate and at the end of the animals life span.

Non-rodents are largely resistant to the phenomenon of peroxisome proliferation and its associated effects such as enzyme induction, hepatomegaly and tumour induction

(see below). Hamsters exhibit weak effects (Lake et al., 1984). On current view, the species differences are largely attributable to the density and functionality of the peroxisome-stimulating (PPAR α) receptor, which in the rat and the mouse is expressed to a particularly high degree and in a complete and functionally active form (Ashby et al., 1994; Bentley et al., 1993; Lee et al., 1995; Cattley et al., 1998; Maloney and Waxman, 1999). In these species, stimulation of the receptors leads to a large number of transcriptions or gene expressions and, morphologically, to proliferation of certain cellular organelles (peroxisomes, mitochondria, endoplasmic reticulum), suppression of apoptosis (Roberts et al., 1998) and an initial (with some substances also continuous) increase in DNA synthesis (Marsman et al., 1988) and mitosis rate after activation of the Kupffer cells (Rose et al., 1997). At all effective doses, the liver is enlarged for a longer period.

Transgenic mice lacking the peroxisome-stimulating receptor (PPAR α) do not exhibit peroxisome proliferation, hepatomegaly or increased DNA synthesis with di(-2-ethylhexyl)phthalate (DEHP; Ward et al., 1998). Testicular and renal damage was less marked in PPAR α deficient mice than in the wild type; this indicates that the species difference was not solely a matter of bioavailability. Furthermore, a highly effective peroxisome proliferator and strong hepatocarcinogen in rats (Wy - 14, 643) did not cause effects in PPAR α knock-out mice (Peters et al., 1997).

The human liver exhibits 1 - 10 % of the functional PPAR α receptor density of mice (Palmer et al., 1998). This is probably the reason for man's lower toxico-dynamic sensitivity, which is also expressed in vitro in liver cell cultures (see below). Experience with fibrate therapies over many years has so far not shown a tumorigenic effect in man.

On the basis of experimental and clinical experience, peroxisome proliferators are currently not classified by IARC as being carcinogenic for man (IARC, 1995/1996). This estimation is mainly shared in more recent publications though in a more differentiated manner and the sense of pronounced quantitative differences (Cattley et al., 1998; Doull et al., 1999; Maloney and Waxman, loc. cit.).

In hepatic cell cultures from the rabbit, guinea pig, marmoset and man, no effects could be observed with DEHP or DINP and other peroxisome proliferators or their active metabolites; the activity of the enzymes palmitoyl-CoA-oxidase and carnitine acetyl transferase remained unchanged, there was no influence on spontaneous or TRGF β 1- induced apoptosis, on DNA synthesis, β -oxidation or the formation of hydroxylauric acid (Elcombe et al., 1997; Ashby et al., 1994; Butterworth et al., 1989; Dirven et al., 1993; Goll et al., 1999; Hasmall et al., 1999).

Reproductive Toxicity:

Developmental effects:

After oral administration of a mixture of 40% DnPP with 60% di-iso-pentylphthalate to Wistar rats in doses of 40, 200 and 1,000 mg/kg from the 6th - 15th day of pregnancy (8 - 10 animals per group; preparation in olive oil) the following results were obtained:

In the top dose all fetuses were resorbed (100% post-implantation loss). The dams showed on day 20 of pregnancy – i.e. 5 days after the end of the application period – ca. 15 and, resp. 12% increases in relative kidney and liver weights. Body-weight gain, when corrected for uterine weights, was normal, so that all reduction in weight gain was due to fetal losses. Relating the only slight maternal to the severe fetal effects, the fetal toxicity is regarded as specific and selective.

No effects were observed at 200 and 40 mg/kg (Hellwig et al., 1997).

Within the frame of a multigeneration study in CD-1 mice following the "continuous breeding protocol" female animals at concentrations of 2.5% DnPP in the diet (~ 4,790 mg/kg/ day) did not give birth when mated to untreated males. This appears to reflect prenatal toxicity at high doses, since the female sexual organs were histologically not affected (Heindel et al., 1989). Since fertility was already mostly impaired at the lowest dose tested in the main study, i.e. 0.5 % in the diet (ca. 760 mg/kg bw d), it can be assumed that prenatal toxicity was also present in this dose group.

Structurally related materials like di-n-butylphthalate (BUA 1987/1993) and di-n-hexylphthalate (Lamb et al., 1987) also show a selective fetal toxicity.

Fertility-reducing effect:

Testicular atrophy has been found in rats with DnPP upon single or 4 consecutive oral administrations of 2,200 mg/kg/day (Foster et al., 1980/1982; Creasy et al., 1983).

Lindström et al. (1988) described a complete loss in fertility in male rats after a single gavage administration of 2,000 mg/kg DnPP. Androgen binding protein (ABP) which is produced and released by Sertoli cells was increased 2 – 3 weeks after the treatment and then reduced from week 4 through 10. Morphologically, the animals showed severe testicular atrophy with empty tubules and abnormal sperm. The same effects were noted at 1,000 mg/kg, but less pronounced.

Within a multi-generation study performed in CD-1 mice according to the "continuous breeding" protocol di-n-pentylphthalate was investigated at concentrations of 0.5, 1.25 and 2.5% in the feed (Heindel et al., 1989; Morrissey et al., 1989). In the highest dose group feed consumption was 8 – 35% higher than in control animals; in the other groups it was normal. Based on the feed consumption the daily doses were estimated to 760, 2,160 and 4,790 mg/kg b.w./day. In the top dose the males lost within 2 weeks 1% of their body weight; at 1.25% they gained 3% in body weight, at 0.5% and in the untreated control group weight gain was 10 and 9%.

In the 1.25 and 2.5% group none of the breeding pairs delivered any litters in the course of the 14 weeks treatment and observation period. In the lowest dose group the number of live pups per litter was reduced from 11.1 ± 0.4 (n = 37) in the control to 1.1 ± 0.5 (n = 4), the number of litters per pair from 4.8 ± 0.1 to 1.3 ± 0.3 and the proportion of pups born alive from 0.98 ± 0.01 to 0.68 ± 0.24 .

As was shown in a crossover mating trial employing concentrations of 2.5% DnPP in the diet, the fertility of both sexes was inhibited; male animals failed to mate or had

insufficient sperm quality in order to achieve copulatory plugs. The weight of kidneys, epididymes, testes, and seminal vesicles were decreased. Prostate weights were reported as normal and liver weights as increased. Histologically, all 20 animals of the top dose showed in 95% of the tubules a severe atrophy. No histological changes were seen in ovaries, oviducts, uterine horn, vagina, liver or kidneys of the treated female mice. Thus, the impairment of fertility in female mice is believed to reflect prenatal toxicity and fetal resorption.

A study on di-n-hexylphthalate employing 0.3, 0.6 and 1.2% in the diet of CD-1 mice (about 500, 1,000 and 2,000 mg/kg bw/day) following the continuous breeding protocol found severe reduction in fertility at 0.6 and 1.2% (cross mating showed both sexes as being affected) and some testicular toxicity at 0.3% (Lamb et al., 1987).

The well investigated and structurally related di-n-butylphthalate (DBP) causes similar damage to the germinal epithelium in the testis and inhibits fertility (BUA 1987/1993; Creasy et al., 1983; Foster et al., 1980; Gray et al., 1982). Sertoli cells exhibit considerable vacuolization of the smooth endoplasmatic reticulum. Germinal epithelium losses are also observed.

In hamsters DBP (2,000 mg/kg/day for 7 days) showed no testicular toxicity; minor changes were noted with DnPP (2,200 mg/kg/day for 9 days): 2/8 animals showed some tubular atrophy with some shedding of spermatids and spermatocytes in > 50% of the tubules whereas in rats the same treatment caused > 90% tubular atrophy in all rats (Gray et al., 1982).

Summary:

Mutagenicity:

Due to the absence of genotoxicity data relating and in accordance with the EC classification criteria, no classification of DnPP is possible (M: -).

Carcinogenicity:

Since no animal data are available on carcinogenicity and since the hepatic effects are of little relevance to man, in accordance with the EC classification criteria it is currently not possible to classify DnPP with in terms of carcinogenicity (C: -).

Reproductive toxicity / developmental damage:

DnPP exerts prenatal toxicity in pregnant mice when given with the feed resulting in doses well below the limit dose.

A selective fetal toxic effect on the rat (with 100 % post-implantation loss) was observed with a 40:60 mixture of DnPP and DIPP at 1,000 mg/kg/day. The dose-response relationship appears to relatively steep since effects were not

observable at 200 mg/kg.

Little difference in toxicological properties is assumed for either components of this mixture and the developmental toxicant di-n-butylphthalate.

There are no indications that DnPP or its primary metabolite MnPP achieve lower bioavailabilities in primates than in rats.

Consequently, in accordance with the EC classification criteria, the DnPP is classified for developmental toxicity into Category (R_E: 2).

Reproductive toxicity / fertility-reducing effect:

Due the findings with di-n-pentylphthalate within the framework of a multi-generation study and other experiments, DnPP exerts testicular atrophy and a fertility impairing effect in dose ranges of relevance for classification.

Also the structurally related compounds DBP and di-n-hexylphthalate inhibit fertility to a similar extent.

DnPP is therefore classified as having a fertility impairing effect (R_F: 2).

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