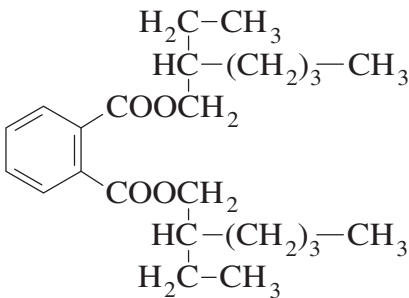


Di(2-ethylhexyl)phthalate (DEHP)

MAK value (1978)	0.62 ml/m³ (ppm) $\hat{=}$ 10 mg/m³
Peak limitation (2002)	Category II, excursion factor 8
Absorption through the skin	–
Sensitization	–
Carcinogenicity (2002)	Category 4
Prenatal toxicity (1989)	Pregnancy risk group C
Germ cell mutagenicity	–
BAT value	–
Synonyms	bis(2-ethylhexyl) phthalate DEHP dioctyl phthalate DOP
Chemical name (CAS)	1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester
CAS number	117-81-7
Structural formula	
Molecular formula	C₂₄H₃₈O₄
Molecular weight	390.57
Melting point	–55 °C
Boiling point	230–235 °C
Vapour pressure at 25 °C	8.6 × 10^{–6} hPa
Density at 20 °C	0.986 g/cm³
log P_{ow}*	3.98–5.45
1 ml/m³ (ppm) $\hat{=}$ 16.206 mg/m³	1 mg/m³ $\hat{=}$ 0.062 ml/m³ (ppm)

* *n*-octanol/water partition coefficient

Di(2-ethylhexyl)phthalate (DEHP) is used widely, in particular as plasticizer in flexible polyvinyl chloride (PVC) products. The amount of DEHP contained in these products can be up to 40 %. DEHP leaches out of PVC products and so can be transferred from packing materials, for example, into foods and medical products (e.g. infusions, transfusions, dialysis systems). As a result of its widespread use and its stability in the environment, DEHP is detectable – in low concentrations – in humans and the environment. However, it must also be remembered that unavoidable contamination during analysis can result in apparent background DEHP levels of about 90 µg/l and MEHP levels (mono(2-ethylhexyl)phthalate) of about 30 µg/l in blood (Kessler *et al.* 2001). High exposures can result, for example, in patients who regularly undergo haemodialysis. Up until the early 1980s, DEHP was the most widely used plasticizer in PVC products for children. Since then, in most countries, DEHP has been replaced by other plasticizers such as di(isononyl)phthalate (IARC 2000).

There is a great deal of literature available on DEHP; this has been summarized in numerous reviews (ATSDR 2000, BUA 1986, 1993, ECB 2000, Fay *et al.* 1999, IARC 2000, WHO 1992). This chapter does not discuss all of the literature in detail. It focuses on more recent studies and literature relevant to the evaluation.

1 Toxic Effects and Mode of Action

After single doses, DEHP is practically non-toxic. The substance does not have irritative or sensitizing effects.

In experimental animals given the substance for longer periods, liver, kidney and testis damage is observed. In a 2-year feeding study, an increased incidence of age-related bilateral aspermatogenesis was found in male F344 rats given doses of 30 mg/kg body weight and day or more (David *et al.* 2000a). In a 13-week feeding study, slight vacuolation of Sertoli cells was seen in male Sprague-Dawley rats given doses of 38 mg/kg body weight and day or more (Poon *et al.* 1997). In a 30-day study reduced testis weights were seen in animals given 50 mg/kg body weight and day or more (Parmar *et al.* 1995). In a 2-generation feeding study with Wistar rats, increased liver weights were seen in the progeny of animals given DEHP doses of 113 mg/kg body weight and day or more. Adverse effects on fertility were only seen after doses above 1000 mg/kg body weight and day (BASF 2001).

In mice given DEHP in the diet for 104 weeks, relevant effects on the kidneys, liver and testes were reported after doses of 100 mg/kg body weight and day or more (David *et al.* 1999, 2000b). Doses of 200 mg/kg body weight and day or more have adverse effects on fertility (Lamb *et al.* 1987).

Malformations have been reported in mice given doses which were not toxic for the dams, 90 mg/kg body weight and day or more (Tyl *et al.* 1988). In rats, developmental toxicity is observed only after maternally toxic doses of over 300 mg/kg body weight and day (NTP 1986, Tyl *et al.* 1988), malformations are observed at doses of over 1000 mg/kg body weight and day (Hellwig *et al.* 1997, Narotsky and Kavlock 1995, Narotsky *et al.* 1995, Peters *et al.* 1997, Ritter *et al.* 1987).

The available data for the genotoxicity of DEHP and its metabolites *in vitro* and *in vivo* do not indicate any clear genotoxic effects of the substances. In various short-term tests for carcinogenicity, DEHP was found to have tumour promoting effects, but not initiating properties. In long-term carcinogenicity studies, DEHP induced liver tumours in rats (Berger 1996, David *et al.* 1999, 2000a, NTP 1982) and mice (David *et al.* 1999, NTP 1982) and mononuclear cell leukaemia in male rats (David *et al.* 1999, 2000a). The incidences of Leydig cell tumours are significantly reduced in two studies (David *et al.* 1999, 2000a, NTP 1982), in one study significantly increased (Berger 1996). Plausible mechanisms that can contribute towards tumour formation are: inhibition of apoptosis, activation of cancer-relevant genes, mobilization of endogenous mitogens, inhibition of intercellular communication or a combination of these mechanisms, which eventually alter the balance between hepatocyte proliferation and apoptosis in favour of the new formation of cells.

2 Mechanism of Action

DEHP induces hepatocellular tumours in the rat and mouse (David *et al.* 1999, 2000a, 2000b, Fay *et al.* 1999, IARC 2000, Kluwe *et al.* 1982, NTP 1982, Rao *et al.* 1990). In addition, in the rat it causes pericytomas and tumours which develop from enterochromaffin cells of the stomach, from Leydig cells, from acinar cells and islet cells of the pancreas (Cattley and Roberts 2000, Fay *et al.* 1999, Rao *et al.* 1990). DEHP does not directly attack DNA; short-term tests for mutagenicity and DNA binding yielded mainly negative results. In two-stage tumorigenesis studies with the rat and mouse in which DEHP was administered in stage 2, DEHP was a tumour promoter (Diwan *et al.* 1985, Huber *et al.* 1996, Kurokawa *et al.* 1988, Sano *et al.* 1999, Ward *et al.* 1986).

In vivo and *in vitro* studies demonstrate that DEHP and its metabolites have both receptor-mediated and receptor-independent effects.

2.1 Receptor-mediated mechanisms

The nuclear receptors which can be activated by DEHP are: PPAR α , γ and δ , (also called PPAR β and NUC1) (Corton *et al.* 2000, Issemann and Green 1990, Lapinskas and Corton 1998). These receptors bind DEHP (or its metabolites), associate then in a second step with the 9-*cis*-retinoic acid receptor (Kliwer *et al.* 1992, Leblanc and Stunnenberg 1995) and in this way become transcription factors. The transcription performance of these factors is modulated by co-activators and co-repressors. The latter amplify or inhibit contact with the transcription apparatus (Gelman *et al.* 1999). The transcription complexes stimulate genes, some of which contain characteristic promoter sequences (e.g. PPRE, peroxisome proliferator responsive element) (Desvergne and Wahli 1999, Reddy and Chu 1996). All three transcription factors bind with base units each containing two 5'-AGGTCA half sequences, which are separated from one another by a nucleotide (so-called DR-1 sequences). The specificity of the effects of individual PPAR

isotypes is determined by the *cis* sequences which flank the base unit; in addition selective co-activator and co-repressor proteins and the specificity of the ligands themselves play a role (Lawrence *et al.* 2001, Rosen and Spiegelman 2001). Another promoter element is NNRE-1 (nuclear receptor regulatory element 1). For PPAR δ a highly specific DNA sequence has been identified (Park *et al.* 2001), although to date no endogenous PPAR δ ligand is known.

The induction of peroxisomes and various peroxisomal enzymes is only one facet of the effects of receptor α and receptor γ stimulators (DeLuca *et al.* 2000). In general there are exogenous ligands which bind preferentially to a certain receptor and others with an overlapping specificity (Kersten *et al.* 2000). DEHP is of the latter kind (Lapinskas and Corton 1998).

As DEHP and some of its metabolites bind to the receptors α , γ and δ , in principle all the effects are to be expected which the physiological and pharmacological ligands for PPAR α , γ and δ trigger. Such ligands are, for example, unsaturated fatty acids, fibrates, eicosanoids, and thiazolidine diones. As far as the activation of PPAR α is concerned, the compounds most extensively investigated to date are nafenopin, a fibrate, and Wyeth-14,643 [(4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio)acetate], a fibrate-like compound. In the field of experimental research, the two substances are used as reference standards and serve as prototypes in the identification of PPAR α -induced effects. DEHP, on the other hand, is less well investigated. As DEHP, however, is an activator of receptors α , γ and δ , in the following text it seems justified to fall back on prototype compounds when biological effects are described which in principle are found with a PPAR activator, but were not analysed specially with the use of DEHP.

The effects induced by receptors α , γ and δ are described below.

2.1.1 Activation of PPAR α

As far as is known, receptor α plays a central role in the genesis of liver tumours in the rat and mouse. Important effects induced by this receptor are e.g.:

- a) the disruption of hepatocyte growth control as a result of increased cell proliferation,
- b) the intensified degradation of fatty acids as a result of the increased expression of peroxisomal, mitochondrial and microsomal enzymes,
- c) the inhibition of apoptosis,
- d) the activation or suppression of, so far unidentified genes.

a) The disruption of hepatocyte growth control as a result of increased cell proliferation
Receptor α ligands, including DEHP, cause in the rat and mouse a rapidly beginning increase in replicative DNA synthesis, which usually returns to normal after a few days, despite the continuous administration of the ligand (exception: Wyeth-14,643). This effect is a response to a primary mitogenic stimulus, not reparative hyperplasia. Hepatomegaly with hypertrophy and hyperplasia develops. The absolute increase in the weight of the organ can amount to twice the original organ weight (Marsman *et al.* 1988). After the higher total cell count of the organ has been reached, normal

hepatocytes evidently lose their sensitivity to the mitogenic effect and they no longer increase in number. Initiated cells, however, remain sensitive (Schulte-Hermann *et al.* 1999).

Receptor α ligands activate proteins and stimulate the expression of genes which regulate the cell cycle and cell proliferation. Detected to date are:

- the genes of the cyclin-dependent kinases 1, 2 and 4, and of cyclin D1 (Peters *et al.* 1998) and the cyclins D1, D3 and E (Macdonald *et al.* 2001),
- the proto-oncogenes *c-myc*, *c-Ha-ras*, *jun-B*, *egr-1*, *c-met*, *c-fos* (Hasmall *et al.* 1997),
- the PCNA gene (proliferating-cell nuclear antigen) (Peters *et al.* 1998),
- the “immediate-early” growth gene *rZFP-37* and the coeruleoplasmin gene (Anderson *et al.* 1999a, Miller *et al.* 1996, Peters *et al.* 1998, Vanden Heuvel 1999),
- protein kinase C (Bojes and Thurman 1994), the transcription factor NF- κ B (Karin *et al.* 2002, Rusyn *et al.* 1998) etc.

The increased cell proliferation can promote tumour development by facilitating during DNA replication the encoding of coincidental DNA matrix damage as a mutation and promoting the clonal expansion of spontaneously initiated cells. PPAR α -mRNA and PPAR α -protein have been detected in humans in the liver, kidneys, heart muscle, skeletal muscle, the testes, but also in other tissue, e.g. in mammary epithelium cells and a breast cancer cell line (Collett *et al.* 2000, Kilgore *et al.* 1997, Mukherjee *et al.* 1994, Palmer *et al.* 1998). PPAR α is expressed in various human organs in smaller amounts than in the mouse and rat, but the receptor is fully functional (Lawrence *et al.* 2001, Mukherjee *et al.* 1994, Roberts *et al.* 2001). Thus mono(2-ethylhexyl)phthalate (MEHP) stimulated the expression and transcription activity of the human receptor α in COS-1 cells which were transfected with PPAR α expression plasmids and reporter plasmids. Under the same experimental conditions MEHP also activated the human receptor γ 2 (Maloney and Waxman 1999). DEHP accelerated the proliferation of human breast cancer cells (Blom *et al.* 1998). A possible connection between receptor α expression and tumour development and progression is visible in the case of the prostate carcinoma. This tumour expresses more receptor α , the more malignant it is (Collett *et al.* 2000).

The receptor α ligand Wyeth-14,643 increased in mice – PPAR α -induced – the activity of steroid-12 α -hydroxylase and thus increased the formation of cholic acid (Hunt *et al.* 2000). PPAR α ligands can also stimulate the expression of genes regulated by the androgen receptor, e.g. the prostate-specific antigen used as tumour marker (Collett *et al.* 2000). The possible activating influence of PPAR α ligands on the expression of other genes dependent on sexual hormones is to be borne in mind.

To summarize, stimulated cell proliferation is an important mechanism on the way to carcinogenesis, but this stimulation alone does not explain the formation of tumours. Other effects of DEHP must also be present, such as e.g. the inhibition of apoptosis, the disruption of intercellular communication and the release of mitogens such as TNF α , interleukin 1 β , and STAT3 (STAT: signal transducer of activated transcription; see the following sections). Decisive are also the properties of the target cells, e.g. their sensitivity for mitogenic effects. In the case of hepatocarcinogenicity, the initiated hepatocyte is regarded as the target cell (Huber *et al.* 1996).

b) The intensified degradation of fatty acids as a result of the increased formation of peroxisomes and mitochondria

PPAR α ligands, including DEHP, induce in hepatocytes of rats and mice an increase in peroxisomes and mitochondria. The increase in these organelles is accompanied by an increase of up to 30 times in the expression of the enzymes of β -oxidation of the fatty acids; also the activity of the enzymes of ω -oxidation of the fatty acids localized in the endoplasmatic reticulum increases (Marsman *et al.* 1988, Reddy and Chu 1996).

Lipases of the organism release from the absorbed DEHP the 2-ethyl-branched alcohol, which is oxidized almost completely to 2-ethylhexanoic acid. The degradation of various 2-methyl-branched (fatty) acid derivatives is catalysed in rodents by peroxisomal pristanoyl-CoA oxidase, trihydroxycoprostanoyl-CoA oxidase and multifunctional enzyme 2 specific for the chiral D form. The corresponding human enzymes are the branched-chain acyl-CoA dehydrogenase (2-methylacyl-CoA dehydrogenase) and the likewise D-specific multifunctional enzyme 2 (Mannaerts and Van Veldhoven 1996). For unknown reasons, none of the enzymes mentioned can be induced by PPAR α agonists, e.g. DEHP. Induced are – but only in rodents – palmitoyl-CoA oxidase and multifunctional enzyme 1, which, however, are not responsible for the degradation of DEHP or its metabolites (in particular 2-ethylhexanoic acid). It seems that the unusual 2-ethyl branching of the 2-ethylhexanoic acid (the compound is present in an R and S form) does not allow sufficiently rapid dehydration for the 2-*trans*-enoyl-CoA derivative to be formed. Also the further degradation steps, namely the hydration of the 2,3 double bond and the dehydration of the 3-hydroxyacyl-CoA formed (catalysed by multifunctional enzyme 2) may be inhibited by the same substituents. The physiologically 2-methyl-branched acids – e.g. 2S,2R-pristanoyl-CoA and 25R-trihydroxycoprostanoyl-CoA – pass through the stages 3-hydroxyacyl-CoA and 3-ketoacyl-CoA rapidly, although 3-hydroxyacyl dehydrogenase accepts only the S isomers, but not the R isomers as substrates (Van Veldhoven and Mannaerts 1999). Decisive is the transformation of the R forms into the S forms by 2-methylacyl-CoA racemase, an accompanying activity of multifunctional enzyme 2. If humans and rodents degraded the mixture of chiral 2-ethyl-branched fatty acids via the same intermediates, such a variety (more than 30!) of metabolites would not be formed from 2R,2S-ethylhexanoic acid as have been identified in the urine of humans and rodents. These metabolites are not products of β -oxidation, but of ω -oxidation, ω -minus-1-oxidation, α -oxidation and other degradation steps untypical of fatty acids.

It has been suggested that fatty acids sustain – in an auto-regulatory manner – lipid homeostasis by means of PPAR α (and γ). Seen in this way, peroxisome increases and the increased expression of ω -hydroxylases of the endoplasmic reticulum can be regarded as an attempt by the cell to overcome degradation difficulties. An only weak increase in peroxisomes was found in human hepatocytes in culture and liver biopsies of patients undergoing fibrate therapy (Ashby *et al.* 1994). Correspondingly, in human hepatocytes fibrates induced only a 2 to 3-fold increase in peroxisomal enzyme activity (Perrone *et al.* 1998). In human glioblastoma cells the α agonist perfluorooctanoate increased the number and volume density of the peroxisomes by 2 to 3.5 times and, together with 9-*cis*-retinoic acid, increased catalase and palmitoyl-CoA oxidase activity (Cimini *et al.* 2000). 4-Phenylbutyrate, which is structurally related to the α agonists fenofibrate and

clofibrate, increased the number of peroxisomes in human fibroblasts by 2.3 times (from 416 to 1014 per cell), but left the expression/protein stability of PPAR α and acyl-CoA oxidase unchanged (Kemp *et al.* 1998). In the myocardium of pigs, a lipid reducer induced marked peroxisome proliferation (Zipper 1997).

In the rat and mouse, however, the intensity of peroxisome proliferation and hepatocarcinogenic effects do not correlate with one another (Marsman *et al.* 1988). Analysis of the effects of the PPAR α ligand dehydroepiandrosterone also showed there to be no correlation between peroxisome proliferation and tumour development. This compound induced peroxisome proliferation mainly in pericentral hepatocytes of the rat; tumours developed, however, almost exclusively in periportal hepatocytes (Beier *et al.* 1997, Metzger *et al.* 1995). A similar uncoupling was observed with the administration of nafenopin. This compound induced in Wistar rats peroxisomal acyl-CoA oxidase in pericentral hepatocytes, cell replication, however, in periportal areas (Grasl-Kraupp *et al.* 1993). Rotenone, a natural insecticide, inhibited in mice hepatocyte proliferation stimulated by the PPAR α agonist Wyeth-14,643, but did not prevent the increase in peroxisomes (Cunningham *et al.* 1995). All in all, peroxisome proliferation alone does not provide a sufficient explanation for the mechanism of tumorigenesis of the receptor α agonists (Melnick 2001).

A possible cause of tumour development is oxidative stress. In the bile of rats given single oral doses of DEHP (1200 mg/kg body weight) an increase in hydroxyl radicals was detected using electron spin resonance; NADPH-oxidase in Kupffer cells was found to be responsible for their production. An increase in free radicals in the liver was detected even before peroxisomal oxidases had been induced (Rusyn *et al.* 2001). In the rat and mouse peroxisomal β -oxidation of fatty acids is increased by more than 20 times and is accompanied by a correspondingly increased hydrogen peroxide production, as the first oxidation step is not linked with the respiratory chain, but reduces molecular oxygen to hydrogen peroxide. The activity of the hydrogen peroxide-detoxifying enzymes, in particular the catalases, is, however, only slightly intensified (< 2-fold) (Conway *et al.* 1989, Reddy and Chu 1996). The hypothesis that metabolites of hydrogen peroxide, e.g. hydroxylperoxide and hydroperoxide radicals, diffused from the peroxisomes into the cell nuclei and there formed 8-hydroxyguanine adducts, which finally caused liver tumours, is deduced from the discrepancy between the hydrogen peroxide-producing and detoxifying capacity: hepatocarcinogenesis as the result of oxidative stress.

The fact that the detoxifying capacity of the peroxisomal catalases, even after intensification of β -oxidation, is greater than the amount of hydrogen peroxide produced by several orders of magnitude (Rose *et al.* 1999a) speaks against this hypothesis. Also quantitative comparison of the effects of Wyeth-14,643 and DEHP shows oxidative stress to be unlikely as the reason for tumour development. Wyeth-14,643 induced in the rat liver many more tumours than the 12-fold amount of DEHP and still the increase in the hydrogen peroxide-detoxifying enzymes remained the same. Even the extent of peroxisome proliferation was the same (Conway *et al.* 1989). In addition, peroxidation products of arachidonic acid (F₂-isoprostanes), the amount of which is a measure of oxidative stress *in vivo*, were not increased after the administration of Wyeth-14,643

(Soliman *et al.* 1997). That DEHP stimulates the expression of repair enzymes which catalyse the excision of oxygen-damaged nucleotides in DNA (e.g. 8-oxoguanine) speaks in favour of the involvement of oxygen radicals in the formation of liver tumours in rodents. These stimulated enzymes include: 8-oxoguanine-DNA glycosylase, apurin endonuclease, DNA-polymerase β etc. (Rusyn *et al.* 2000).

There is no clear causal relationship between the effects of DEHP on peroxisomes and lipid metabolism and hepatocarcinogenesis. In addition, after administration of PPAR α activators tumours (occasionally) developed in the rat even in those cells for which to date no peroxisome proliferation could be detected, e.g. enterochromaffine cells of the stomach, Leydig cells, acinar cells of the pancreas (see Cattley and Roberts 2000) and perisinusoidal cells (Ito cells) of the liver (Stroebel *et al.* 1995).

At present there are, therefore, no data available that prove that peroxisome proliferation is an obligatory step towards tumour development (Melnick 2001). PPAR α -activating substances can evidently activate cancer-relevant genes without peroxisome proliferation taking place.

Unlike in the rat and mouse, hepatocytes produce hardly any increase in peroxisomes in humans after incubation with α agonists (Reddy and Chu 1996). Various explanations for this have been given, among others that human hepatocytes contain only about a tenth of the amount of PPAR α (Palmer *et al.* 1998) – with a greater variability in the amount of mRNA (Tugwood *et al.* 1996). The decisive reason could, however, be that the genes of peroxisomal β -oxidation in humans are regulated in a different way to those of the rat and mouse, i.e. their promoter sequences react differently or require other *cis*- and *trans*-activating factors (co-activators or co-repressors) (Hasmall *et al.* 1999, 2000).

PPAR α is active in the human liver, as the reduction in triglycerides and the increase in HDL cholesterol therapeutically produced by fibrates is activated by the same receptor α of the hepatocytes which in rodents causes peroxisome proliferation (Reddy and Chu 1996, Staels *et al.* 1997). In addition, the genes whose expression is increased (or reduced) carry the characteristic promoter sequences mentioned above (Staels *et al.* 1997, Vu-Dac *et al.* 1995). In humans, fibrates increase the expression of the lipoprotein-lipase gene and the apolipoprotein-A-I and A-II genes; they reduce the expression of the apolipoprotein-C-III gene (Hertz *et al.* 1995, Kersten *et al.* 2000). In addition, α agonists (e.g. fenofibrate) in human hepatocytes stimulated the mRNA synthesis of hydroxymethylglutaryl-CoA synthase and carnitine-palmitoyl transferase – a sign of the activation of receptor α – while they slowed down the mRNA synthesis of apolipoprotein C-III, which is also evidence of the activity of receptor α (Lawrence *et al.* 2001).

c) The inhibition of apoptosis

The prototypical PPAR α ligand nafenopin inhibits spontaneous apoptosis and that induced by DNA damage or TGF β -1 in the hepatocytes of the rat, mouse, hamster and guinea pig (James and Roberts 1996, James *et al.* 1998) [TGF β -1: transforming growth factor β -1, a negative regulator of liver growth]. Noteworthy about this result is that the inhibition of apoptosis was observed even in the hepatocytes of those animal species which are not capable of induced peroxisome proliferation or increased DNA synthesis, namely the hamster and guinea pig. Evidently, in the four animal species mentioned, the inhibition of apoptosis and peroxisome proliferation are independent of one another. In

hamsters and guinea pigs, in addition, the inhibition of apoptosis is uncoupled from induced DNA synthesis (James and Roberts 1996).

The suppression of apoptosis has the effect that damaged or spontaneously initiated cells, which are normally eliminated by apoptosis, remain in the organism, where, stimulated by mitogens, they can develop into tumours. The mechanism of apoptosis inhibition has not been investigated, but there is evidence that nafenopin slows down two signal cascades of apoptosis induction: the p53-dependent cascade, which results from DNA damage, and the p16/p27-dependent cascade, which is activated by TGF β -1 (Gill *et al.* 1998). The involvement of protein kinase C in apoptosis inhibition has been demonstrated (Roberts 1996). DEHP acts as hepatic tumour promoter in the mouse and rat (Huber *et al.* 1996). This effect was correlated with apoptosis inhibition of initiated and preneoplastic cells (Schulte-Hermann *et al.* 1999).

d) Suppression of or increase in the expression of genes so far unidentified

Administration of the PPAR α activator Wyeth-14,643 for two weeks led in the mouse, a species with marked peroxisome proliferation, to changes in the amounts of 49 liver proteins; 47 of these decreased, 2 increased. In the hamster, which produces only slight peroxisome proliferation, under the same experimental conditions changes were observed in 35 liver proteins; 27 decreased, 8 increased. There was no overlapping between the changed proteins of the mouse and hamster, except for an unidentified protein and the bifunctional enzyme (enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase). The latter was increased in the mouse to 5 times the reference value and in the hamster to 7 times the corresponding reference value (Giometti *et al.* 1998). Comparison makes it clear that α agonists in the mouse and hamster – independent of peroxisome proliferation – depress and intensify gene activity and change the lifespan of mRNA or proteins. Most of the affected genes are, however, still unknown.

In rat hepatocytes the prototypical PPAR α ligand nafenopin caused within 6 to 24 hours both an increase in DNA synthesis and also changes in the expression of 32 proteins, some of which were peroxisome-independent (Chevalier *et al.* 2000). The complex changes in expression show that PPAR α activators influence numerous different metabolic and regulatory functions. In the same cell system also tumour necrosis factor α (TNF α) increased replicative DNA synthesis and modulated the expression of 11 of the 32 proteins mentioned above. The overlapping of the reaction pattern suggests similarities in signal transduction, and DEHP does indeed mobilize the tumour necrosis factor (see Section 2.2 Receptor-independent mechanisms).

2.1.2 Activation of PPAR γ

Also this receptor, which under physiological conditions regulates the differentiation and function of fat cells and modulates the growth of epithelial cells in different organs (Lowell 1999, Rosen and Spiegelman 2001, Rosen *et al.* 1999), may play a role in carcinogenesis. γ Agonists increase the expression, for example, of genes belonging to the family of carcinoembryonal antigens (Gupta *et al.* 2001). Colorectal tumours express these antigens to a large extent. PPAR γ is activated by DEHP or its metabolites (Lapinskas and Corton 1998).

The γ activators troglitazone and rosiglitazone facilitate the formation of intestinal tumours in the so-called *min* mouse (*min*: multitude of intestinal neoplasms) (Lefebvre *et al.* 1998, Saez *et al.* 1998). *Min* mice serve as a model for studying colon carcinogenesis. They have only one functional *APC* gene; its loss leads to the development of colon tumours.

On the other hand, the activation of PPAR γ can also lead to the inhibition of tumour growth. The γ agonist troglitazone thus caused differentiation of human colon cancer cells and delayed the growth of colon cancer transplantation tumours (Sarraf *et al.* 1998). In the presence of γ activators, liposarcomas from patients and human breast cancer cell lines produced terminal differentiation, which was associated with a less malignant and more slowly growing phenotype (Demetri *et al.* 1999, Elstner *et al.* 1998, Mueller *et al.* 1998).

The contradictory effects of the γ activators are still not understood. A model was put forward (Park *et al.* 2001) in which it was suggested that PPAR δ promotes cell proliferation in human cells, while PPAR γ inhibits this and induces apoptosis. Both receptors, according to the suggestion, compete for the same binding partner, the 9-*cis*-retinoic acid receptor, and thus produce an equilibrium. Disturbance of this equilibrium – by an increase in PPAR δ activity or a reduction in PPAR γ activity – would facilitate the formation of colon tumours. Colon carcinomas, liposarcomas and mammary tumours in humans can, however, express both receptors (PPAR γ and δ) to a great extent (Demetri *et al.* 1999, Elstner *et al.* 1998, Mueller *et al.* 1998, Sarraf *et al.* 1998) and nevertheless evidently grow better. Activation of PPAR γ by DEHP could increase this growth advantage e.g. by promoting the synthesis of the carcinoembryonal antigens (see above).

2.1.3 Activation of PPAR δ

This receptor is expressed in practically all cells. PPAR $\delta^{-/-}$ mice are smaller than the control animals and, after topical administration of the tumour promoting phorbol ester TPA, produced increased hyperplastic skin reactions. At the same time, in the skin the mRNAs of the cell cycle/cell proliferation proteins CDK-1, CDK-4, cyclin B1 and PCNA were increased (Peters *et al.* 2000). DEHP activates PPAR δ (Lapinskas and Corton 1998). The PPAR δ gene is repressed – together with the *c-myc* and cyclin D1 gene – by the (intact) APC/ β -catenine protein. This protein is defective in hereditary colon carcinomas and often also in sporadic colon carcinomas in humans as a result of gene mutations, so that it does not fulfil its repressor function and as a result PPAR δ is expressed to a greater extent.

The defective mutation of the *APC* gene is regarded as the central genetic change in the transformation of a normal colon mucosa cell into the colon cancer cell (Kinzler and Vogelstein 1996). In accordance with this interpretation, the specific inactivation of the PPAR δ gene in a human colon carcinoma cell line stopped these cells from being able to form tumours in the nude mouse (Park *et al.* 2001). Preventive administration of aspirin derivatives delays the formation of colon tumours, e.g. by suppressing the binding of PPAR δ to its specific promoter sequences (Park *et al.* 2001).

On the basis of these data it seems plausible that DEHP activates receptor δ in an unphysiological way and thus stimulates defective functions, which promote the malignant condition. This is the effect of the loss of the APC repressor function: it allows the over-expression of receptor δ .

2.2 Receptor-independent mechanisms

The following are examples of receptor-independent mechanisms which are associated with carcinogenesis:

- a) the activation of Kupffer cells by the mobilization of NF- κ B and the release of mitogens, e.g. TNF α and interleukin-1 β (Chen *et al.* 2002). These activate e.g. mitosis in hepatocytes and at the same time suppress apoptosis. The activation of Kupffer cells is independent of whether they have the PPAR $\alpha^{+/+}$ or PPAR $\alpha^{-/-}$ genotype (Hasmall *et al.* 2001).
- b) morphological transformation, disturbance of intercellular communication (Cruciani *et al.* 1997) and chromosome aberrations.

a) Activation of Kupffer cells by the mobilization of NF- κ B and the release of mitogens
MEHP and other PPAR α activators initiate a signal cascade (Bojes and Thurman 1996, Rose *et al.* 2000). This evidently begins with the inhibition of acyl-CoA synthase and the resulting increase in free fatty acids in Kupffer cells in the liver. Fatty acids activate Ca⁺⁺-dependent and phospholipid-dependent protein kinase C (it is not known which of the 12 isotypes) and stimulate their *de novo* synthesis. Protein kinase C phosphorylates NADH oxidase, which in turn produces superoxide. MEHP stimulated the superoxide production of the Kupffer cells just as effectively as the prototypical PPAR α ligand Wyeth-14,643; purified hepatocytes, however, did not form superoxide under the same conditions (Rose *et al.* 1999b). In Kupffer cells superoxide activates the transcription factor NF- κ B; this induces the production of TNF α . TNF α diffuses into neighbouring hepatocytes, stimulates mitosis there and inhibits apoptosis. Mitosis stimulation and apoptosis inhibition require the involvement of PPAR α -regulated functions (Hasmall *et al.* 2001). The mechanism of this co-operation is unknown.

Inhibitors of the activation of Kupffer cells (methyl palmitate, glycine) suppressed the release of TNF α and hepatocyte proliferation, but not the proliferation of the peroxisomes. That means that mitogenic and peroxisome-proliferating stimuli use separate signal routes and are independent of one another (Rose *et al.* 2000).

The model described was modified on the basis of results of further experiments (Anderson *et al.* 2001). According to the authors PPAR α agonists activate NF- κ B in Kupffer cells directly or via PPAR α . NF- κ B promotes the synthesis and secretion of interleukin-1 β , a mediator of hepatocyte proliferation. Interleukin-1 β stimulates the specific interleukin receptor IL1R1 of the hepatocytes via a paracrine pathway. As a result STAT3 is mobilized [signal transducer of activated transcription], a regulator of

the cell cycle, which is expressed to a greater extent in many kinds of tumours in humans. STAT3 causes oncogenic effects in hepatocytes by activating the cell cycle genes *c-myc*, *p21*, *cyclin D1* and the antiapoptotic *bcl-x_L* gene. As certain PPAR α agonists in hepatocytes at the same time induce the synthesis of PPAR γ 1 (Anderson *et al.* 2001), other tumorigenic effects are to be expected, which take place via the β -catenine mechanism (see above).

b) Morphological transformation, disturbance of intercellular communication and chromosome aberrations

In the transformation test with cells of the Syrian hamster, DEHP increased the incidence of morphologically transformed colonies (Mikalsen and Sanner 1993, Tsutsui *et al.* 1993). The increase was more pronounced after the addition of S9 mix (Tsutsui *et al.* 1993). MEHP, a phase I metabolite of DEHP, which was found in the urine of persons exposed to DEHP, had no effects. On the other hand, in the presence of S9 mix MEHP induced both cell transformation and chromosome aberrations in cells of the Chinese hamster (Tsutsui *et al.* 1993). Evidently, MEHP needs to be oxidized or cleaved to produce phthalic acid and 2-ethylhexanol in order to have this effect. 2-Ethylhexanol is then present as a mixture of both chiral forms (as in the esters). In rodents the 2*S* form can be degraded by β -oxidation, the 2*R* form as such cannot (Van Veldhoven and Mannaerts 1999).

In primary hepatocyte cultures from humans and the rat, the prototype compound Wyeth-14,643 caused the formation of micronuclei. In addition, in rat hepatocytes chromosome aberrations were increased by up to 10 times (Hwang *et al.* 1993). DEHP also reduced intercellular communication by 30 % (Mikalsen and Sanner 1993). The results clearly show that genetic effects of DEHP, which are evidently receptor-independent, can contribute towards the carcinogenicity of the compound.

Other effects caused by PPAR α agonists

In the literature, other effects which are caused by PPAR α agonists are described:

- DEHP caused changes in the thyroid gland of rats, which are regarded as signs of hyperactivity: a reduction in follicle size, an increase in prismatic/conical follicle cells (Howarth *et al.* 2001)
- DEHP disrupted the hormonal equilibrium during the sexual differentiation of the male rat, induced hyperplasia of Leydig cells and reduced the testosterone level (Parks *et al.* 2000)
- DEHP suppressed in the rat preovulatory oestradiol synthesis and ovulation (Davis *et al.* 1994)
- Wyeth-14,643 caused hypomethylation of the *c-myc* gene of mouse liver and at the same time an increase in proliferation (Ge *et al.* 2001)
- α agonists (Wyeth-14,643, fenofibrate) reduced the activity of 11 β -hydroxysteroid dehydrogenase, type 1, in mouse liver by deactivating the corresponding gene. As a result of this expression inhibition, neither cortisone nor 11-dehydrocorticosterone can be transformed into their active forms cortisol and corticosterone. The effects are:

cortisol deficiency, reduction of plasma glucose and insulin levels and increase in insulin sensitivity (antidiabetic effect), prevention of hepatic gluconeogenesis. The effects demonstrate a lack of adaptation to a hunger situation (Hermanowski-Vosatka *et al.* 2000)

2.3 Summary of the different mechanisms

What is known about the multiple effects of DEHP makes it clear that it has numerous “main effects” which are induced by different receptors and are also receptor-independent.

Some more recent publications attribute the role of a central mechanism to the peroxisome proliferation induced by DEHP (Doull *et al.* 1999, IARC 2000). This is contradicted by the fact that according to more recent insights peroxisome proliferation cannot be regarded as an obligatory transition stage for the formation of tumours. In addition, peroxisome proliferation does not explain tumour formation, or only very unsatisfactorily (Melnick 2001). Plausible mechanisms that can contribute towards tumour formation are: inhibition of apoptosis, activation of cancer-relevant genes, mobilization of endogenous mitogens, inhibition of intercellular communication or a combination of these mechanisms, which eventually alter the balance between hepatocyte proliferation and apoptosis in favour of the new formation of cells.

The fact that human hepatocytes display only slight peroxisome proliferation should not lead one to assume that other effects of DEHP are excluded. Not excluded are a) other receptor α -regulated effects, b) receptor β and γ -induced effects and c) effects which are receptor-independent. The finding that α agonists (fibrates) in human hepatocytes stimulate the expression of genes of lipid and cholesterol metabolism is clear evidence of peroxisome-independent effect mechanisms, which DEHP can also induce. As the receptors α , β and γ regulate basic biological functions, possible target cells of DEHP are not only hepatocytes, but also other body cells.

There are several peroxisome-independent effects for which a plausible link with carcinogenesis can be established: e.g. the inhibition of apoptosis, the activation of endogenous mitogens, the increased expression of genes of cell proliferation and the stimulation of initiated cells to produce clonal growth. As already mentioned, apoptosis inhibition in hepatocytes was observed also in those animal species which, like the human hepatocyte, display only slight peroxisome proliferation and are regarded as “refractory” to PPAR α agonists.

Thus, there is much to suggest that the activation of cancer-relevant genes is one of the foremost effects of DEHP, while genotoxic effects, above all at low exposure concentrations, play a subordinate role.

3 Toxicokinetics and Metabolism

There are numerous studies available on the absorption, metabolism and excretion of DEHP in humans and animals (IARC 2000, WHO 1992), which are summarized below.

3.1 Absorption

3.1.1 Humans

At the workplace, inhalation is the main route of absorption of DEHP. Ingestion of DEHP is possible via food (in particular after the leaching out of DEHP from plastic packaging). In dialysis patients intravenous absorption of DEHP also plays a role. Dermal absorption is slow (IARC 2000).

3.1.2 Animals

In rats given single oral doses of ^{14}C -labelled DEHP (2.9 mg/kg body weight) at least 50 % was absorbed; after 7 days 42 % had been excreted with the urine and 14 % with the bile. In the stomach DEHP is rapidly hydrolysed to MEHP, which is absorbed around 16 times better than DEHP. Cynomolgus monkeys hydrolyse DEHP in the stomach less effectively than the rat and mouse. Also in marmosets the substance is absorbed in the gastrointestinal tract to a lesser extent than in rodents (WHO 1992).

Seven days after single dermal applications of ^{14}C -labelled DEHP (61.5 mg/kg body weight) to the shaved backs of F344 rats, about 5 % of the applied amount of DEHP had been excreted (WHO 1992). Strips of PVC film (15 cm²), which contained ^{14}C -labelled DEHP, were attached to the shaved backs of rats for 24 hours in 2 experiments. In one experiment, the urine and faeces were collected over 7 days after the PVC film was removed, in the other experiment the animals were killed after the PVC film was removed. In both studies, the amount of DEHP that escaped from the plastic film was very small (0.064 % and 0.126 %). Of this amount 75 % could easily be removed from the site of application; the rest remained at the site of application or was absorbed. From this study absorption rates of 0.239 and 0.242 $\mu\text{g}/\text{cm}^2$ and hour were calculated (Deisinger *et al.* 1998).

3.2 Distribution

DEHP given to rats orally is distributed in the body mainly as MEHP. DEHP which had not been metabolized was found in the liver only after higher doses (> 500 mg/kg body weight). After intravenous injection of radioactively labelled DEHP, radioactivity was found in the liver, lungs and spleen (WHO 1992).

3.3 Metabolism

Detailed descriptions of metabolism can be found in IARC (2000), WHO (1992) and Doull *et al.* (1999). DEHP is cleaved mainly at an ester group by lipases from the pancreas. The breakdown products are mono(2-ethylhexyl)phthalate (MEHP) and 2-ethylhexanol. 2-Ethylhexanol is oxidized further to 2-ethylhexanoic acid. The further degradation of MEHP takes place at the chain ends of the remaining monoester group as follows (Figure 1):

- 1) Hydroxylation of the terminal CH₃ groups of both the ethyl side chains and the hexyl side chains (ω -oxidation) or oxidation of the last but one C atom of the hexyl chain (ω -1-oxidation)
- 2) Oxidation of the hydroxyl groups to carboxylic acid (ω -oxidation) or to the ketone (ω -1-oxidation)
- 3) Shortening of the side chain as a result of α or β -oxidation of the carboxylic acid from ω -oxidation

In rats about 80 % of the administered DEHP is hydrolysed (WHO 1992). Hydrolysis is catalysed primarily by pancreatic lipases. In laboratory animals the levels of pancreatic lipases can differ greatly depending on the species and strain. Lower enzyme levels are found in the liver, blood and other tissue (IARC 2000).

After administration of DEHP, MEHP and the metabolites mono(5-carboxy-2-ethylpentyl)phthalate (V), mono(5-ethyl-5-oxo-hexyl)phthalate (VI) and mono(2-ethyl-5-hydroxyhexyl)phthalate (IX) were detected in the urine of humans, the monkey, the rat and the mouse (see Figure 1). In humans and the monkey the metabolites were usually in glucuronidized form; in the rat no glucuronidized metabolites were found. The quantitative amounts differed markedly between the studies (see IARC 2000). MEHP and the metabolites mono(5-ethyl-5-oxohexyl)phthalate (VI) and mono(2-ethyl-5-hydroxyhexyl)phthalate (IX) are held primarily responsible for the peroxisome proliferating activity (IARC 2000).

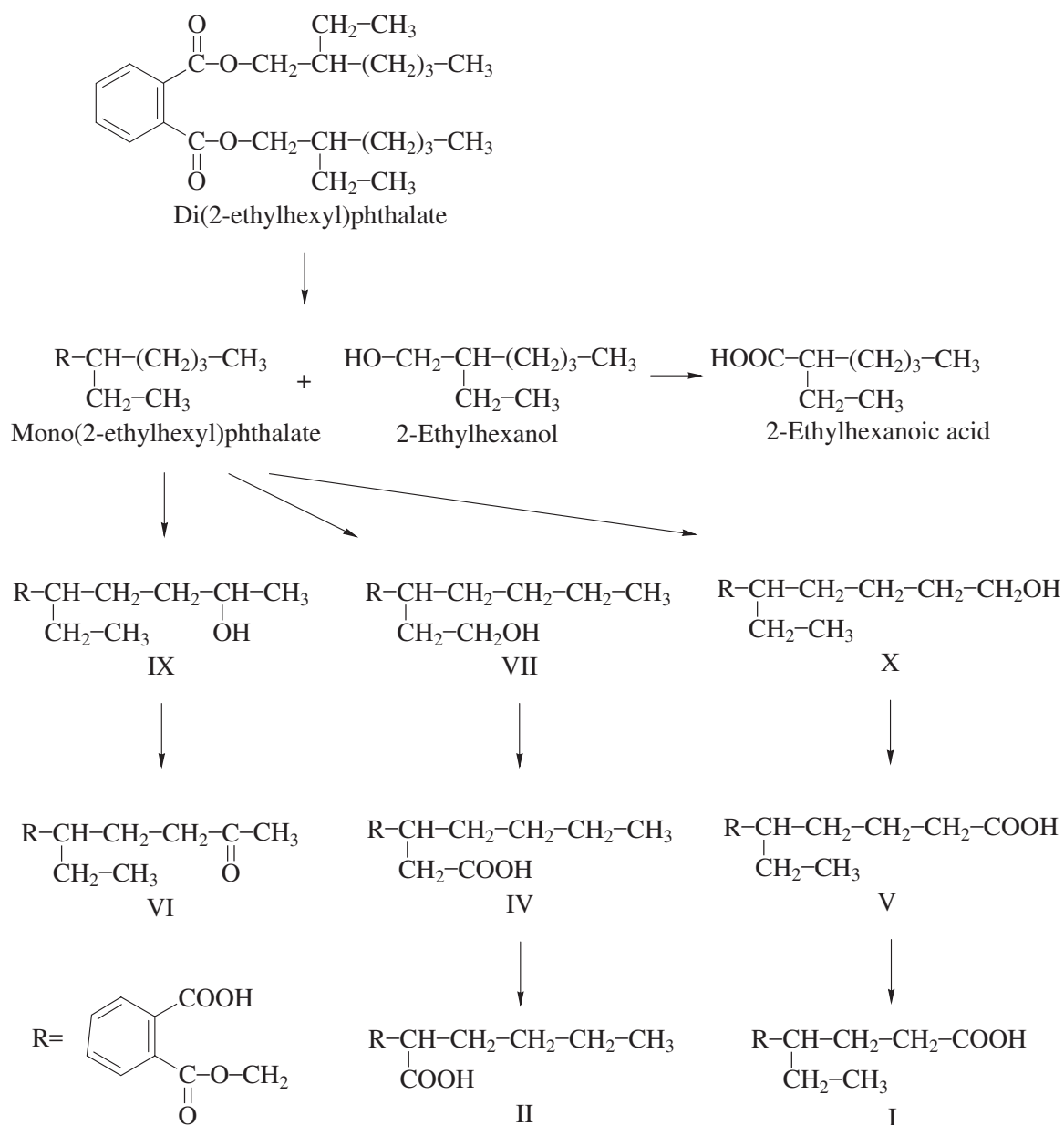


Figure 1. Metabolism of di(2-ethylhexyl)phthalate (IARC 2000).

I Mono(4-carboxy-2-ethylbutyl)-phthalate, II Mono(2-carboxyhexyl)phthalate,
 IV Mono(2-(carboxymethyl)hexyl)phthalate, V Mono(5-carboxy-2-ethylpentyl)phthalate,
 VI Mono(2-ethyl-5-oxohexyl)phthalate, VII Mono(2-(2-hydroxyethyl)hexyl)phthalate,
 IX Mono(2-ethyl-5-hydroxyhexyl)phthalate, X Mono(2-ethyl-6-hydroxyhexyl)phthalate

3.4 Elimination

After two persons were given single oral doses of 5000 or 10000 mg DEHP, within 24 hours about 4.5 % of the administered amount was eliminated with the urine. After two persons were given single oral doses of 30 mg DEHP, 11 % to 15 % were eliminated with the urine within 24 hours. The half-time for elimination with the urine was given as about 12 hours. Administration of doses of 10 mg to two persons four times a day did not yield evidence of accumulation. Between 11 % and 33 % of the dose was excreted daily with the urine (IARC 2000).

4 Effects in Humans

4.1 Single exposures

Ingestion of 10000 mg DEHP caused in one person slight gastritic disturbances and moderate diarrhoea, while ingestion of 5000 mg DEHP was tolerated without symptoms by a second person (Shaffer *et al.* 1945).

4.2 Repeated exposures

4.2.1 Patients

Among 27 haemodialysis patients with terminal kidney failure, three cases were described with non-specific hepatitis. The PVC containers used released DEHP concentrations of 10 to 20 mg/l perfusate. The symptoms and signs of hepatitis disappeared quickly when DEHP-free containers were used (WHO 1992).

A correlation between the DEHP, MEHP, phthalic acid or 2-ethylhexanol concentrations in serum after dialysis and the occurrence of uraemic pruritus was not recognizable (IARC 2000).

In 3 premature babies who were artificially ventilated with PVC respiratory tubes, unusual lung changes were observed during the fourth week after birth. The authors attribute these changes to DEHP (WHO 1992). 18 of 28 newborn babies with breathing difficulties received “extracorporeal membrane oxygenation”, intervention which resulted in exposure to DEHP of up to 2 mg/kg body weight over 3 to 10 days (mean DEHP concentrations in plasma of 8 µg/ml). Various clinical, liver, lung and heart function parameters were unchanged (IARC 2000).

4.2.2 Workers

In workers of a factory producing DEHP (see Section 4.6), no changes were observed in blood lipids, the activities of liver enzymes in serum and routine haematological investigations. The concentrations of DEHP in the air were, however, below 0.16 mg/m^3 (IARC 2000, WHO 1992).

The peripheral nervous system was investigated in 54 male workers of a PVC processing factory. The workers were exposed mainly to DEHP, di(isodecyl)phthalate and butylbenzylphthalate. Three exposure groups were formed with mean phthalate concentrations of 0.1, 0.2 and 0.7 mg/m^3 . Signs and symptoms of peripheral nervous disorders were found in some workers, but these did not correlate with the exposure concentrations. None of the workers were found to have symptoms of work-related obstructive lung disease, nor did the results of lung function tests correlate with exposure. Some biochemical parameters correlated significantly, however, with exposure. A slight reduction in the haemoglobin levels thus correlated with increasing exposure duration and exposure in previous years. With increasing exposure duration also the α -1-antitrypsin levels were slightly increased and the serum immunoglobulin A levels increased with exposure in previous years (WHO 1992).

4.3 Local effects on skin and mucous membranes

After local application of DEHP to the skin of volunteers, no or only weak irritation of the skin was reported (see Section 4.4).

4.4 Allergenic effects

Two cases of work-related asthma were described, which were probably caused by DEHP. One employee with moderate unspecific bronchial hyperreactivity who processed a plastics mixture which contained 64.9 % polyvinyl chloride and 32.5 % DEHP was tested in a 30-minute bronchial provocation test (BPT) with $100 \mu\text{l}$ DEHP heated to 120°C (theoretical maximum chamber concentration 14.1 mg/m^3). A decrease of 19 % was determined in the forced expiratory volume in the first second (FEV_1) and after another 2 hours of 15 %. At the same time, the mean forced expiratory flow rate (MEF_{25-75}) was decreased by 52 % and 35 %. Previous administration of 40 mg disodium cromoglycate prevented a reaction in the BPT (Brunetti and Moscato 1984). Another employee, who was exposed to the vapour of a glue containing DEHP during the production of bottle stoppers, produced an immediate reaction with a decrease in the FEV_1 after 10 minutes of 38 % in a workplace-related provocation test. The exact composition of the glue was not given, but the authors stated that in the work area only DEHP could be detected, in a concentration of 0.14 to 0.64 mg/m^3 (Cipolla *et al.* 1999). Findings which indicate immunological genesis were not discovered in either case, and both groups of authors regard a specific, but non-immunological mechanism as probable.

Other studies which reported asthma symptoms or eye and nose symptoms in connection with the presence in buildings of MEHP and 2-ethylhexanol hydrolysis products formed from DEHP (Norbäck *et al.* 2000, Oie *et al.* 1997, Wieslander *et al.* 1999), likewise did not yield evidence of airway sensitization.

There is only an incompletely documented communication available about a possible contact allergic reaction to DEHP in the plastic of headphones of a radar technician. The employee produced a simple reaction to 2 % DEHP in petrolatum in the patch test after 2 and 4 days (Walker *et al.* 2000). 30 workers with dermatitis from a polyvinyl chloride factory, 30 workers without dermatitis and 30 control persons were tested in the patch test with “dioctyl phthalate” (structure, purity and concentration not stated). In 1/30 workers with dermatitis, 4/30 workers without dermatitis and 0/30 control persons the results were positive (ECB 2000, Vidovic and Kansky 1985).

None of 839 patients who were tested with components of plastics and glues in addition to the standard series in the patch test reacted to 2 % “dioctyl phthalate” (Tarvainen 1995).

In the clinics of the Information Network of Departments of Dermatology, 599 patients were tested with 5 % DEHP in petrolatum between the years 1992 and 2000. After 72 hours, one patient produced a simple reaction, another a 2+ reaction. In addition, 3 questionable reactions and one follicular reaction were documented (IVDK 2001).

In a patch test with application of undiluted DEHP to the backs of 23 volunteers for 7 days and repeated application 10 days later on the same site, no erythema or other reactions were described (Shaffer *et al.* 1945). DEHP was applied occlusively to the upper arm of a group of 200 volunteers (100 men, 100 women) for 48 hours. 15 days later provocation was carried out under the same conditions. Reactions (erythema) were observed in only 2/100 men. According to the authors, the low incidence, low severity and the course of the reactions do not indicate a sensitizing potential for the test substance (ECB 2000). In a human repeated insult patch test (HRIPT), 0.2 ml DEHP (purity over 99 %) was applied occlusively to the upper arm of 128 volunteers, three times a week, for 24 hours, over three weeks. During the induction treatment and after the provocation treatment which was carried out after a 10 to 17-day interval, no signs of irritation or sensitization were observed in any of the 104 volunteers. The other 24 persons broke off the study, according to the authors, for reasons that were not connected with the tests (Medeiros *et al.* 1999).

The triggering of contact urticaria in one patient was attributed to contact with PVC on cotton gloves and work clothes. In the scratch test, immediate reactions to 2 % DEHP in petrolatum and to extracts containing DEHP (5 % in petrolatum) from clothing were observed (Sugiura *et al.* 2000, 2002).

4.5 Genotoxicity

Ten workers employed for 10 to 30 years in the production of DEHP were not found to have increased chromosomal aberrations. The exposure to DEHP was very low with concentrations of 0.09 to 0.16 mg/m³ (Thiess and Fleig 1978).

4.6 Carcinogenicity

In a mortality study, 221 workers from a company producing DEHP were investigated between 1940 and 1976. 135 of the 221 workers were employed after 1965; from 1966 on, DEHP was produced in completely closed systems. Among the workers there were 8 deaths, compared with 15.9 (for the city of Ludwigshafen and Rheinland Palatinate) and 17.0 (nationally) expected deaths. Among the workers with an exposure duration of more than 20 years, there was one worker with a pancreatic tumour (0.13 expected) and one worker with a bladder papilloma (0.01 expected) (IARC 2000). The IARC draws attention to the fact that most of the workers were employed after the exposure concentrations had been drastically reduced and that the study design is not adequately described.

There are no data available for the toxic effects of DEHP on reproduction.

5 Animal Experiments and *in vitro* Studies

5.1 Acute toxicity

Studies of the acute toxicity of DEHP are summarized in Table 1.

With 4-hour LC₅₀ values for DEHP of more than 10000 mg/m³ in the rat (ECB 2000) and oral LD₅₀ values of more than 20000 mg/kg body weight in the rat (Hodge 1943, Krauskopf 1973, NTP 1982, Shaffer *et al.* 1945), mouse (Hodge 1943, Krauskopf 1973), guinea pig (Krauskopf 1973) and rabbit (Shaffer *et al.* 1945), DEHP is practically non-toxic. Even the dermal LD₅₀ in the rabbit is more than 20000 mg/kg body weight (ECB 2000, Shaffer *et al.* 1945). LD₅₀ values for intraperitoneal administration are also more than 10000 mg/kg body weight in the rat (Shaffer *et al.* 1945, Singh *et al.* 1972) and mouse (Calley *et al.* 1966, Gesler and Kartinos 1970, Lawrence *et al.* 1975).

After oral administration of DEHP to rats, diarrhoea (Hodge 1943) and swelling in the liver and kidneys (Shaffer *et al.* 1945) were reported.

Table 1. Studies of the acute toxicity of DEHP

Route of absorption	Species	LD ₅₀	References
Inhalation	rat	> 23670 mg/m ³ (1 h)	ECB 2000
		> 10620 mg/m ³ (4 h)	ECB 2000
		> 600 mg/m ³ (6 h)	ECB 2000
Oral	rat	> 20000 mg/kg body weight	NTP 1982
		33700 mg/kg body weight (34.3 ml/kg)	Krauskopf 1973
		30600 mg/kg body weight	Shaffer <i>et al.</i> 1945
		> 34000 mg/kg body weight	Hodge 1943
	mouse	22200 mg/kg body weight (22.6 ml/kg)	Krauskopf 1973
		> 128000 mg/kg body weight	Hodge 1943
		33400 mg/kg body weight (34 ml/kg)	Krauskopf 1973
		26300 mg/kg body weight	Krauskopf 1973
Dermal	rabbit	33900 mg/kg body weight	Shaffer <i>et al.</i> 1945
		> 19600 mg/kg body weight (> 20 ml/kg)	Shaffer <i>et al.</i> 1945
		24500 mg/kg body weight (25 ml/kg, 24 h occlusive)	ECB 2000
Intraperitoneal	rat	> 49150 mg/kg body weight (> 50 ml/kg)	Singh <i>et al.</i> 1972
		30700 mg/kg body weight	Shaffer <i>et al.</i> 1945
	mouse	37770 mg/kg body weight	Lawrence <i>et al.</i> 1975
		> 7500 mg/kg body weight	Gesler and Kartinos 1970
		14190 mg/kg body weight	Calley <i>et al.</i> 1966

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

In a 28-day inhalation study, groups of 27 male and 17 female **Wistar rats** were exposed to DEHP concentrations of 0, 10, 50 or 1000 mg/m³. For male animals the resulting daily doses were estimated to be 0, 2.3, 11 and 230 mg/kg body weight, and for female animals 0, 3.6, 18 and 360 mg/kg body weight. At the highest concentration increased lung weights and histological changes in the lung (increased foam cells, thickening of the alveolar septa) and increased liver weights were detected. No effects on the testes were detected. **The NOEL (no observed effect level) was found to be 50 mg/m³ (11 and 18 mg/kg body weight and day)** (Klimisch *et al.* 1992).

5.2.2 Ingestion

There are numerous studies for DEHP with repeated oral administration; these studies are reviewed e.g. in ATSDR (2000), IARC (2000) and WHO (1992). In the present documentation only those studies are taken into consideration which are most important for the setting of a MAK value, in particular more recent studies with medium-term and long-term administration of the substance.

In experiments with rats and mice, after long-term administration damage to the liver (peroxisome proliferation, increased liver weights, hypertrophy, necrosis, *spongiosis hepatis*, adenomas, carcinomas), testes (decreased testis weights, degeneration of the seminiferous tubules, tubular atrophy, aspermatogenesis) and kidneys (changed kidney weights, chronic progressive nephropathy) were observed. Studies with PPAR α knock-out mice showed that liver toxicity is observed only in wild-type mice which express PPAR α . Kidney and testicular toxicity, however, occurred in both wild-type and (to a lesser extent) in PPAR α knock-out mice (Ward *et al.* 1998) (Table 2).

In one study, **rats** (strain not stated) were given doses of DEHP of 2.1 mg/kg body weight three times a week for 3, 6, 9 or 12 months. The kidneys from 4 animals from each group were histopathologically examined after 3, 6, 9 and 12 months exposure. Only after 12 months were cystic changes found in the kidneys of the animals and were often accompanied by inflammation. There was a statistically significant reduction in creatinine clearance. Cystic changes in the kidneys and reduced creatinine clearance are, according to the authors, normally observed in old rats (Crocker *et al.* 1988). This study provides evidence that age-related changes in the kidneys of rats can develop earlier with the administration of DEHP. As a result of the study design (e.g. only one dose group, a small number of animals, no details of the strain, incomplete histopathology), this study cannot, however, be included in the evaluation of the MAK value.

In a 13-week study, a markedly increased incidence of severe vacuolation of Sertoli cells was found in male **Sprague-Dawley rats** relative to in the control animals, after doses of DEHP of 500 mg/kg diet or more (38 mg/kg body weight and day). In the control group 0/10 animals were affected, in the lowest dose group (5 mg/kg diet) 4/10 animals (severity 0.2/4), in the next highest dose group (50 mg/kg diet) 4/10 animals (severity 0.5/4), in the next dose group (500 mg/kg diet) 7/10 animals (severity 1.0/4) and in the highest dose group (5000 mg/kg diet) 9/10 animals (severity 2.4/4). After doses of 5000 mg/kg diet (375 mg/kg body weight and day), tubular atrophy in the testes, increased liver weights, an increased peroxisome count, hepatocellular hypertrophy and necrosis, increased kidney weights, a reduced erythrocyte count, reduced haemoglobin and increased albumin were also found. In female animals, effects on the liver and kidneys were likewise detected. As Sertoli cell vacuolation is to be observed also with other phthalates (Hild *et al.* 2001, Jones *et al.* 1993, Poon *et al.* 1997) and other substances which cause testicular toxicity (Inomata *et al.* 2000, Shoda *et al.* 2001), and is preceded by morphological changes, the authors regard the vacuolation observed after doses of DEHP of 500 mg/kg diet as early adverse effects. **The authors therefore regard 50 mg/kg diet (3.7 mg/kg body weight and day) as the NOAEL** (Poon *et al.* 1997). The authors, however, give no historical control data for the vacuolation of Sertoli cells. In addition, instead of the usual Bouin's solution, Zenker

fluid was used as the fixative for the testes and epididymis. As, however, there was a marked increase in the incidence and severity of Sertoli cell vacuolation after doses of DEHP of 500 mg/kg diet, and as this is observed as an early change also with other phthalates and other substances which cause testicular toxicity, the authors' assessment has been adopted and the given NOAEL has been included in the evaluation of the MAK value.

Table 2. Selected medium-term and long-term studies with DEHP in the rat, mouse and monkey

Species, strain, number of animals	Exposure	Findings	References
rat , groups of 4, no other details	3, 6, 9, 12 months , 2.1 mg/kg body weight, 3×/week, p.o.	2.1 mg/kg body weight: <u>kidneys</u> (cystic kidney changes, creatinine clearance decreased); scope of the study: body weight gains, kidney histology, creatinine clearance	Crocker <i>et al.</i> 1988
rat, SD , groups of 10 ♂, 10 ♀	13 weeks , 0, 5, 50, 500, 5000 mg/kg diet (♂: 0, 0.4, 3.7, 38, 375 mg/kg body weight and day; ♀: 0, 0.4, 4.2, 42, 419 mg/kg body weight and day)	3.7 mg/kg body weight: NOAEL ≥ 38 mg/kg body weight ♂: <u>testes</u> (slight vacuolation of Sertoli cells) 375 mg/kg body weight ♂: <u>testes</u> (marked vacuolation of Sertoli cells, testicular atrophy), <u>liver</u> (absolute and relative weights increased, peroxisome count increased, hepatocellular hypertrophy and necrosis), <u>kidneys</u> (relative weights increased), <u>haematology/clinical chemistry</u> (erythrocyte count decreased, haemoglobin decreased, albumin increased); ♀: <u>liver</u> (absolute and relative weights increased, peroxisome count increased, hepatocellular hypertrophy and necrosis), <u>kidneys</u> (relative weights increased, not significant), <u>clinical chemistry</u> (albumin/globulin increased, cholesterol decreased)	Poon <i>et al.</i> 1997
rat, SD , groups of 15 ♂, 15 ♀	17 weeks , 0, 2000, 10000, 20000 mg/kg diet (♂: 0, 143, 737, 1440 mg/kg body weight and day; ♀: 0, 154, 797, 1414 mg/kg body weight and day)	≥ 143 mg/kg body weight ♂, ♀: <u>liver</u> (absolute and relative weights increased), <u>testes</u> (slight damage, not significant), <u>pituitary gland</u> (castration cells: 1/15) ≥ 737 mg/kg body weight ♂: body weight gains decreased, food consumption decreased, <u>testes</u> (absolute and relative weights decreased, atrophy), <u>kidneys</u> (relative weights increased), <u>brain</u> (relative weights increased), <u>haematology</u> (haemoglobin decreased); ♀: body weight gains decreased, food consumption decreased, <u>kidneys</u> (relative weights increased), <u>haematology</u> (haemoglobin decreased) 1440 mg/kg body weight ♂: <u>pituitary gland</u> (relative weights increased); ♀: <u>pituitary gland</u> (absolute weights increased)	Gray <i>et al.</i> 1977

Table 2. continued

Species, strain, number of animals	Exposure	Findings	References
rat, SD, 520 ♂	102 weeks, 0, 200, 2000, 20000 mg/kg diet (0, 7, 70, 700 mg/kg body weight and day)	<p>≥ 7 mg/kg ♂: <u>testes</u> (atrophy, inhibition of spermatogenesis; data not described)</p> <p>≥ 70 mg/kg ♂: <u>liver</u> (peroxisomal enzyme activities increased, peroxisome proliferation)</p> <p>700 mg/kg body weight ♂: body weight gains decreased</p>	Ganning <i>et al.</i> 1991
rat, F344, groups of 10 ♂, 10 ♀	13 weeks, 0, 1600, 3100, 6300, 12500, 25000 mg/kg diet (0, 80, 160, 320, 630, 1250 mg/kg body weight and day)	<p>320 mg/kg body weight: no effects</p> <p>630 mg/kg body weight ♂: <u>testes</u> (atrophy)</p> <p>1250 mg/kg body weight ♂, ♀: body weight gains decreased</p>	NTP 1982
rat, F344, groups of 50 ♂, 50 ♀	103 weeks, 0, 6000, 12000 mg/kg diet (♂: 0, 322, 674 mg/kg body weight and day; ♀: 0, 394, 774 mg/kg body weight and day)	<p>≥ 322 mg/kg body weight ♂: body weight gains slightly decreased; ♂, ♀: <u>liver</u> (clear cell changes in the liver, adenomas and carcinomas)</p> <p>674 mg/kg body weight ♂: <u>testes</u> (degeneration of the seminiferous tubules), <u>pituitary gland</u> (hypertrophy); ♀: body weight gains slightly decreased</p>	Kluwe 1986, Kluwe <i>et al.</i> 1982, NTP 1982
rat, F344, groups of at least 50 ♂, 50 ♀	104 weeks, 0, 100, 500, 2500 12500 mg/kg diet (♂: 0, 6, 29, 147, 789 mg/kg body weight and day; ♀: 0, 7, 36, 181, 938 mg/kg body weight and day)	<p>at 6 mg/kg body weight: no relevant effects (♀: mononuclear leukaemia increased, not in higher dose groups)</p> <p>≥ 6 mg/kg body weight ♂: <u>kidneys</u> (age-related mineralization of the renal papillae increased)</p> <p>≥ 29 mg/kg body weight ♂: <u>testes</u> (age-related bilateral aspermatogenesis increased)</p> <p>≥ 147 mg/kg body weight ♂: <u>kidneys</u> (absolute and relative kidney weights increased, mineralization, chronic nephropathy), <u>liver</u> (palmitoyl-CoA oxidase activity increased, absolute and relative liver weights increased, <i>spongiosis hepatis</i>, adenomas and carcinomas), <u>lungs</u> (relative lung weights increased), mononuclear leukaemia increased; ♀: <u>liver</u> (palmitoyl-CoA oxidase activity increased, absolute and relative liver weights increased)</p>	David <i>et al.</i> 2000a

Table 2. continued

Species, strain, number of animals	Exposure	Findings	References
		<p>789 mg/kg body weight ♂: body weight gains decreased, food consumption decreased, survival slightly decreased (not significant), <u>testes</u> (absolute and relative testis weights decreased, bilateral aspermatogenesis), <u>kidneys</u> (tubule pigmentation), <u>liver</u> (Kupffer cell pigmentation), <u>pancreas</u> (proliferative lesions, adenomas), pituitary gland (castration cells), <u>brain</u> (brain weights increased), <u>clinical chemistry</u> (urea and albumin increased, globulin decreased), haematology (erythrocyte count decreased, haematocrit decreased, haemoglobin decreased);</p> <p>♀: body weights and food consumption decreased, <u>liver</u> (Kupffer cell pigmentation, adenomas and carcinomas increased), <u>kidneys</u> (absolute and relative kidney weights increased), <u>brain</u> (relative brain weights increased)</p>	
mouse, PPARα^{+/-} (Sv/129), PPARα^{-/-} , 5 ♀ of each	24 weeks , 12000 mg/kg diet (1800 mg/kg body weight and day)	<p>1800 mg/kg body weight: PPARα^{+/-}: body weight gains decreased, mortality (100 % after 16 weeks), <u>liver</u> (relative weights increased, histopathological changes), <u>kidneys</u> (relative weights increased, cysts, histopathological changes), <u>testes</u> (relative weights decreased, histopathological changes)</p> <p>PPARα^{-/-}: body weight gains, mortality unchanged, no changes in the liver, <u>kidneys</u> (histopathological changes), <u>testes</u> (histopathological changes)</p>	Ward <i>et al.</i> 1998
mouse, B6C3F₁ , groups of 10 ♂, 10 ♀	13 weeks , 0, 800, 1600, 3100, 6300, 12500 mg/kg diet (0, 100, 200, 400, 800, 1600 mg/kg body weight and day)	<p>100 mg/kg body weight ♀: body weight gains decreased</p> <p>400 mg/kg body weight ♂: body weight gains decreased</p>	NTP 1982
mouse, B6C3F₁ , groups of 50 ♂, 50 ♀	103 weeks , 0, 3000, 6000 mg/kg diet (♂: 0, 672, 1325 mg/kg body weight and day; ♀: 0, 799, 1821 mg/kg body weight and day)	<p>≥ 672 mg/kg body weight ♂: <u>liver</u> (adenomas and carcinomas increased); ♀: body weight gains decreased</p> <p>1325 mg/kg body weight ♂: <u>kidneys</u> (chronic nephropathy), <u>testes</u> (degeneration of the seminiferous tubules); ♀: <u>liver</u> (adenomas and carcinomas increased)</p>	Kluwe 1986, Kluwe <i>et al.</i> 1982, NTP 1982

Table 2. continued

Species, strain, number of animals	Exposure	Findings	References
mouse, B6C3F₁ , groups of at least 60 ♂, 60 ♀	104 weeks , 0, 100, 500, 1500 6000 mg/kg diet (♂: 0, 19, 99, 292, 1266 mg/kg body weight and day; ♀: 0, 24, 117, 354, 1458 mg/kg body weight and day)	<p>≥ 19 mg/kg body weight: NOAEL</p> <p>≥ 99 mg/kg body weight ♂: <u>liver</u> (palmitoyl-CoA oxidase activity increased, absolute liver weights increased, adenomas and carcinomas increased), <u>kidneys</u> (relative kidney weights decreased), <u>testes</u> (relative testis weights decreased); ♀: <u>liver</u> (palmitoyl-CoA oxidase activity increased)</p> <p>≥ 292 mg/kg body weight ♂: <u>liver</u> (relative liver weights increased), <u>kidneys</u> (absolute kidney weights decreased, chronic progressive nephropathy increased), <u>testes/epididymis</u> (absolute testis weights decreased, bilateral hypospermia increased, immature or abnormal sperms in the epididymis); ♀: <u>liver</u> (relative liver weights increased; adenomas and carcinomas increased)</p> <p>1266 mg/kg body weight ♂: body weight gains decreased, survival decreased, <u>liver</u> (hepatocellular pigmentation, cytoplasmatic eosinophilia, chronic inflammation), <u>kidneys</u> (absolute kidney weights decreased, chronic progressive nephropathy increased), <u>lungs</u> (relative lung weights increased), <u>brain</u> (relative brain weights increased); ♀: <u>liver</u> (absolute liver weights increased, hepatocellular pigmentation, cytoplasmatic eosinophilia), <u>kidneys</u> (kidney weights decreased, chronic progressive nephropathy increased), <u>uterus</u> (uterus weights decreased)</p>	David <i>et al.</i> 1999, 2000b
monkey, marmoset , groups of 4 ♂, 4 ♀	13 weeks , 0, 100, 500, 2500 mg/kg body weight, p.o.	<p>≥ 500 mg/kg body weight ♂: <u>liver</u> (peroxisome volumes increased, P450 activity increased); ♀: <u>liver</u> (P450 activity increased)</p> <p>2500 mg/kg body weight ♂: body weight gains decreased; scope of the study: clinical chemistry, haematology, testosterone, oestradiol, cholecystokinin, hepatic peroxisomal enzymes, P450, analysis of zink in the testes, examination of all organs under the light microscope, examination of the liver and testes with the electron microscope</p>	Kurata <i>et al.</i> 1998

p.o: peroral, SD: Sprague-Dawley

In an earlier 17-week study with **Sprague-Dawley rats**, liver weights were increased in both sexes after the lowest dose of 2000 mg/kg diet (143 mg/kg body weight and day) and doses above this. In male animals slight damage to the testes was detected (not

significant), and in one animal castration cells were found in the pituitary gland. The effects on the testes were significant after the next highest dose of 10000 mg/kg diet (about 737 mg/kg body weight and day) and above. In addition, the weights of the kidneys and brain were increased and the haemoglobin level in blood decreased (Gray *et al.* 1977).

In a 102-week study of the effects of DEHP on the liver in **Sprague-Dawley rats**, peroxisome proliferation was observed after the middle dose of 2000 mg/kg diet (about 70 mg/kg body weight and day) and doses above that. At the low dose of 200 mg/kg diet (7 mg/kg body weight and day) only testicular atrophy and inhibition of spermatogenesis were reported (Ganning *et al.* 1991). As the findings in the testes were not described, it is not possible to make an evaluation and the findings in the testes cannot be included in the evaluation of the MAK value.

In an earlier 13-week study of the NTP with **F344 rats**, no effects were observed up to doses of 6300 mg/kg diet (320 mg/kg body weight and day). Testicular atrophy was increased after doses of 12500 mg/kg diet and more (about 630 mg/kg body weight and day) (NTP 1982).

In the subsequent long-term study with **F344 rats**, body weight gains were slightly reduced in the male animals after the lowest dose of 6000 mg/kg diet (about 360 mg/kg body weight and day) and doses above that. Changes in clear cells and adenomas and carcinomas of the liver were observed in rats of both sexes. At the high dose of 12000 mg/kg diet (720 mg/kg body weight and day), in the male animals hypertrophy of the pituitary gland and degeneration of the seminiferous tubules in the testes were observed. In the female animals body weight gains were slightly reduced (Kluwe 1986, Kluwe *et al.* 1982, NTP 1982).

In a more recent 2-year study with **F344 rats**, at the lowest dose of 100 mg/kg diet (about 6 mg/kg body weight and day) the incidence of mononuclear leukaemia was increased. As such effects were not found at the higher doses, this finding is regarded as coincidental. After doses at this level and higher, however, also a dose-dependent increase in the incidence of mineralization of the renal papilla was described in male animals. This is usually an age-related effect. It cannot be excluded, however, that the effect is substance-related as DEHP accelerates the development of some age-related effects. At doses of 500 mg/kg diet (30 mg/kg body weight and day), the incidence of age-related bilateral aspermatogenesis was increased. After doses of 2500 mg/kg diet (160 mg/kg body weight and day) and above, in the male animals effects were found in the liver (increased peroxisomal enzyme activity, increased liver weights, *spongiosis hepatis*, liver adenomas and carcinomas), kidneys (increased kidney weights, mineralization, chronic nephropathy), lungs (increased lung weights), and mononuclear leukaemia was detected. In the female animals, effects were observed in the liver (increased peroxisomal enzyme activity, increased liver weights, *spongiosis hepatis*). After doses of 12500 mg/kg diet (about 850 mg/kg body weight and day) and above, in the male animals reduced food consumption, reduced body weight gains and slightly reduced survival were observed. At this dose, also effects on the testes (reduced testis weights, bilateral aspermatogenesis) were found. In addition to other effects on the blood count (decreased erythrocyte count), increased relative brain weights, castration cells in the pituitary gland, Kupffer cell and renal tubule pigmentation, and proliferative lesions

and adenomas in the pancreas were observed. In the female animals, food consumption and body weight gains were likewise reduced, and effects on the liver (Kupffer cell pigmentation, liver adenomas and carcinomas) and kidneys (increased kidney weights) were observed (David *et al.* 2000a). The authors conclude from the findings in the recovery group that the effects on the liver are (to a certain extent) reversible, but the effects on the testes and kidneys, and the leukaemia, are not (David *et al.* 2001). On the basis of the 2-year study, the authors give 500 mg/kg diet to be the NOAEL (David *et al.* 2000a). As at this dose, however, the incidence of age-related bilateral aspermatogenesis was increased and at the next lowest dose of 100 mg/kg diet (6 mg/kg body weight and day) the incidence of age-related mineralization of the renal papillae was increased and amplified, and these age-related effects are evidently amplified by the administration of DEHP, these effects cannot be ignored in the evaluation of the MAK value. **Thus, 6 mg/kg body weight and day is regarded as the dose which has slight effects.**

In an earlier 13-week study by the NTP with **B6C3F₁ mice**, body weight gains were decreased in the female animals after the lowest dose of 800 mg/kg diet (100 mg/kg body weight and day) and doses above that, and in the male animals after doses of 1600 mg/kg diet (400 mg/kg body weight and day) and above (NTP 1982). In the subsequent long-term study, body weight gains were reduced in the female animals after the lowest dose of 3000 mg/kg diet (about 730 mg/kg body weight and day) and doses above that, and in the male animals liver tumours were found. At the high dose of 6000 mg/kg diet (about 1600 mg/kg body weight and day), effects on the kidneys and testes were observed in male animals, and liver tumours in the female animals (NTP 1982).

In a more recent study with **B6C3F₁ mice**, after administration of DEHP in doses of 100 mg/kg diet (about 20 mg/kg body weight and day) for 104 weeks, in male animals the incidence of adenomas of the liver was slightly increased (23 %), but the increase was not statistically significant. In the mouse, an increase in liver tumours is often observed with the increasing age of the animals. In the study control group, the incidence of liver adenomas (11 %) was greatly below the incidence in historical controls (27 %). Also at this dose there was no correlation with biochemical or histological parameters. This was therefore probably not a biologically relevant increase in the incidence of adenomas. On the other hand, at the next highest dose of 500 mg/kg diet (about 100 mg/kg body weight and day), clear effects on the liver (increased absolute liver weights, liver adenomas and carcinomas), kidneys (reduced relative kidney weights) and testes (reduced relative testis weights) were observed in male animals. In animals of both sexes, peroxisomal enzyme activity was increased. After doses of 1500 mg/kg diet (about 300 mg/kg body weight and day) and above, chronic progressive nephropathy, bilateral hypospermia in the testes and immature or abnormal sperms in the epididymis were also observed. In female animals increased liver weights and liver adenomas and carcinomas were observed at this dose and above. At the highest dose of 6000 mg/kg diet (1300 mg/kg body weight and day), in addition to other changes, body weight gains and survival were reduced in male animals (David *et al.* 1999, 2000b). **From this study a NOAEL of 100 mg/kg diet (20 mg/kg body weight and day) was deduced.**

Groups of 4 male and 4 female **monkeys** (marmoset) were given gavage doses of DEHP of 0, 100, 500 or 2500 mg/kg body weight and day for 13 weeks. After doses of 500 mg/kg body weight and day or more, in the male animals the volume of peroxisomes had increased 1.3-fold and in animals of both sexes the activity of CYP450 was increased. At 2500 mg/kg body weight and day, body weight gains were reduced in the male animals. Apart from the slight increase in peroxisome volume in the male animals, no effects on the liver or testes were observed even with the electron microscope (Kurata *et al.* 1998). As a result of methodological shortcomings (e.g. small number of animals, the “negative” histopathological findings are not described), this study cannot be included in the evaluation of the MAK value.

5.3 Local effects on skin and mucous membranes

The irritative effects of DEHP on skin and mucous membranes have been investigated in several studies (ECB 2000). In studies carried out according to OECD guideline 404, DEHP was not found to be irritative to the skin and mucous membranes (ECB 2000).

5.4 Allergenic effects

In a modified Buehler test with guinea pigs, carried out in accordance with EU guidelines 92/69/EEC, DEHP was not found to have sensitizing effects. Induction treatment was carried out on days 1, 8 and 15 of the experiment with 0.5 ml DEHP (purity not stated; occlusive application on the left shoulder for 6 hours). Skin reactions were determined 30 minutes and 24 hours after removal of the substance. Two weeks after the last induction treatment, provocation was carried out also with 0.5 ml DEHP (occlusive application on the right shoulder for 6 hours). Skin reactions were not observed either during the induction treatment or 24, 48 and 72 hours after removal of the substance used for provocation (ECB 2000).

It is also reported that in a Magnusson and Kligman maximization test DEHP was not found to have sensitizing effects (no other details; ECB 2000). “Dioctylphthalate” (DEHP) was used as the test substance; the purity of the substance was not given.

In an intracutaneous and a non-occlusive epicutaneous test, for induction 5 guinea pigs of both sexes were given either subcutaneous injections of a 0.1 % (w/v) mixture of various phthalic acid diesters in paraffin, or the substance was applied to the backs of the animals, 3 times a week for 3 weeks. Provocation, 10 days after the last induction treatment, was carried out with the same concentration and form of administration. The result was read 1, 24 and 48 hours later. After the induction treatment slight symptoms of irritation were observed; after provocation, however, there were no sensitization reactions (ECB 2000). As a result of the mixture of different phthalic acid diesters used and the low substance concentration, this study is only of limited meaningfulness.

In studies with BALB/c mice, DEHP (and MEHP) were found to have an adjuvant effect in the sense that they stimulated the formation of ovalbumin-specific IgG₁ (Larsen *et al.* 2001).

It cannot be excluded that DEHP under certain conditions can undergo pyrolysis and thus form phthalic anhydride (Madsen *et al.* 1988, McNeill and Memetea 1994, Pfäffli 1986, Vainiotalo and Pfäffli 1990), which has sensitizing effects (see the chapter “Phthalic anhydride” in Volumes 7 and 11 of the present series).

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

5.5.1.1 Inhalation

In a 28-day inhalation study (see Section 5.2) groups of 27 male and 17 female **Wistar rats** were exposed to DEHP concentrations of 0, 10, 50 or 1000 mg/m³. For male animals the resulting daily doses were estimated to be 0, 2.3, 11 and 230 mg/kg body weight, and for female animals 0, 3.6, 18 and 360 mg/kg body weight. At the highest dose, liver and lung weights were increased. Testicular effects were not observed. The NOAEL was found to be 50 mg/m³. After the treated male animals were mated with untreated females, no effects on fertility were detected (Klimisch *et al.* 1992).

5.5.1.2 Ingestion

Studies of the testicular toxicity of DEHP after oral administration can be found in Table 3, fertility studies (multi-generation studies) in Table 4.

In one study, young prepubescent or young adult **Long-Evans rats** were given DEHP for 14 or 28 days. After exposure for 14 days, inhibition of the testosterone production in Leydig cells was observed in pubescent animals after doses of 10 mg/kg body weight and day or more. Exposure for 28 days, however, increased the testosterone production in the Leydig cells of pubescent rats. In the adult animals no effects were observed up to the highest dose of 200 mg/kg body weight and day (Akingbemi *et al.* 2001). As adverse effects on the testes were still not detected in this dose range after administration of DEHP to rats (animals likewise pubescent at the beginning of exposure) for 2 years, the reported effects on the testosterone production were regarded as adaptive.

In a study with **Wistar rats** given DEHP for 30 days, testis weights were decreased and testicular enzymes changed after the lowest dose of 50 mg/kg body weight and day and doses above this (Parmar *et al.* 1995; Table 3). On the other hand, in a 2-generation study with Wistar rats, effects on fertility, tubular atrophy of the testes and toxic effects (mortality, reduced body weight gains) were observed only after high doses of 9000 mg/kg diet (1088 mg/kg body weight and day). In this study **the NOAEL for fertility was given as 3000 mg/kg diet (340 mg/kg body weight and day)**. For developmental toxicity the authors deduced a NOAEL of 1000 mg/kg diet (113 mg/kg body weight and day); at this dose, however, liver weights were slightly increased in the offspring (BASF 2001; Table 4). In a mechanistic study of DEHP, the formation of reactive oxygen species was increased after Wistar rats were given gavage doses of 1000

or 2000 mg/kg body weight and day for 7 days. Correspondingly, the levels of glutathione and ascorbic acid in the testes were decreased, and apoptosis of the spermatocytes and testicular atrophy were observed (Kasahara *et al.* 2002).

Table 3. Studies of the testicular toxicity of DEHP in rats after oral administration

Species, strain, number of animals	Exposure	Findings	References
rat, Long Evans, groups of 10 ♂, 21, 35 or 62 days old	14 days, PND 21–34 (JA1), PND 35–48 (JA2); 28 days, PND 21–48 (JB), PND 62–89 (JC); 0, 1, 10, 100, 200 mg/kg body weight and day, p.o.	1 mg/kg body weight: no effects ≥ 10 mg/kg body weight: 14-day exposure of pubescent (JA2) rats: serum testosterone not changed (<i>in vivo</i>), inhibition of 17β-hydroxysteroid dehydrogenase, inhibition of the testosterone production in Leydig cells (<i>in vivo/in vitro</i> determination); 28-day exposure of prepubescent/pubescent rats (JB): increased serum testosterone and luteinizing hormone (<i>in vivo</i>), increased testosterone production in Leydig cells (<i>in vivo/in vitro</i> determination) 100 mg/kg body weight: 14-day exposure of prepubescent (JA1) rats: serum testosterone not changed (<i>in vivo</i>), inhibition of the testosterone production in Leydig cells (<i>in vivo/in vitro</i> determination); 28-day exposure of young adult male rats (JC): no such effects	Akingbemi <i>et al.</i> 2001
rat, Wistar, groups of 5 ♂, 25 days old	30 days, 0, 50, 100, 250, 500 mg/kg body weight and day, p.o.	≥ 50 mg/kg body weight: absolute (significant) and relative (not significant) testis weights decreased, changes in testicular enzymes: γ-glutamyl transpeptidase increased, lactate dehydrogenase increased, sorbit dehydrogenase decreased ≥ 100 mg/kg body weight: relative testis weights (significantly) decreased, CYP450 decreased ≥ 250 mg/kg body weight: histopathological changes in testes, β-glucuronidase increased, acid phosphatase decreased	Parmar <i>et al.</i> 1995

Table 3. continued

Species, strain, number of animals	Exposure	Findings	References
rat, Wistar, groups of at least 5 ♂, 4–5 weeks old	7 days, 0, 1000, 2000 mg/kg body weight and day, p.o.	≥ 1000 mg/kg body weight: testes (weights decreased, glutathione peroxidase increased, catalase increased, glutathione decreased, ascorbic acid decreased, reactive oxygen species increased); liver (weights increased) 2000 mg/kg body weight: kidney weights decreased	Kasahara <i>et al.</i> 2002
rat, F344, groups of 48 ♂, 63–70 days old	13 days, 0, 330, 1000, 3000 mg/kg body weight and day, p.o.; diet: normal (Zn _{normal}), high zinc (Zn _{high}) or low zinc (Zn _{low})	0 mg/kg body weight: body weight gains decreased, gonad weights decreased (Zn _{low}) 330 mg/kg body weight: no effects 1000 mg/kg body weight: slight testicular atrophy (Zn _{normal} ; not significant) 3000 mg/kg body weight: body weight gains decreased (Zn _{normal} ; not with Zn _{high}), slight testicular atrophy (Zn _{normal} ; significant)	Agarwal <i>et al.</i> 1986a
rat, F344, groups of 24 ♂, 60 days old	60 days, 0, 320, 1250, 5000, 20000 mg/kg diet (0, 17.5, 69.2, 284, 1156 mg/kg body weight and day)	69 mg/kg body weight: no effects 284 mg/kg body weight: body weight gains decreased, gonad weights decreased, testosterone decreased, luteinizing hormone increased, follicle stimulating hormone increased 1156 mg/kg body weight: testicular degeneration, Zn level in testes decreased, sperm parameters in epididymis decreased, litter size decreased after mating with untreated ♀	Agarwal <i>et al.</i> 1986b
rat, F344, groups of 50 ♂, 42 days old	103 weeks, 0, 6000, 12000 mg/kg diet (0, 322, 674 mg/kg body weight and day)	322 mg/kg body weight: body weight gains slightly decreased 674 mg/kg body weight: degeneration of the seminiferous tubules in the testes	NTP 1982 (see Table 2)
rat, F344, groups of at least 50 ♂, 42 days old	104 weeks, 0, 100, 500, 2500, 12500 mg/kg diet (0, 6, 29, 147, 789 mg/kg body weight and day)	6 mg/kg body weight: NOAEL ≥ 29 mg/kg body weight: age-related bilateral aspermatogenesis increased 789 mg/kg body weight: absolute and relative testis weights decreased, bilateral aspermatogenesis, castration cells in the pituitary gland	David <i>et al.</i> 2000a (see Table 2)
rat, SD, groups of 5 ♂, 3 days old	1x, 20, 100, 200, 500 mg/kg body weight, p.o., offspring examined 4 days after birth	20 mg/kg body weight: no effects ≥ 100 mg/kg body weight: Sertoli cell proliferation decreased, cyclin D2 expression decreased ≥ 200 mg/kg: multinuclear gonocytes	Li <i>et al.</i> 2000

Table 3. continued

Species, strain, number of animals	Exposure	Findings	References
rat, SD, groups of 15 ♂, 1, 2, 3, 6, 12 weeks old	5 days, 0, 10, 100 1000, 2000 mg/kg body weight and day, p.o.	≥ 10 mg/kg body weight: palmitoyl-CoA oxidase activity increased ≥ 100 mg/kg body weight: relative liver weights increased ≥ 1000 mg/kg body weight: body weight gains decreased (1, 2, 3-week-old animals), relative testis weights decreased (1, 2, 3, 6-week-old animals), number of Sertoli cells decreased (only 1-week-old animals), loss of spermatocytes (2- and 3-week-old animals), loss of spermatids and spermatocytes, Zn concentration in the testes decreased (6- and 12-week-old animals) 2000 mg/kg body weight: survival decreased (unweaned pups), body weight gains decreased (6- and 12-week-old animals)	Dostal <i>et al.</i> 1987b, 1988
rat, SD, groups of 5 ♂, 21 days old	1–14 days, 2000 mg/kg body weight and day, p.o.	2000 mg/kg body weight: testicular changes: apoptosis, necrosis, atrophy, zinc depletion (secondary effect resulting from testicular toxicity)	Park <i>et al.</i> 2002
rat, SD, groups of 15 ♂, 35 days old	6 weeks, 0, 10000, 20000 mg/kg diet (0, 750, 1500 mg/kg body weight and day)	750 mg/kg body weight: enlarged livers 1500 mg/kg body weight: testicular atrophy, aspermatogenesis (effects on the testes can be avoided by the administration of vitamin C or E)	Ishihara <i>et al.</i> 2000
rat, SD, groups of 10 ♂, 42 days old	13 weeks, 0, 5, 50, 500, 5000 mg/kg diet (0, 0.4, 3.7, 38, 375 mg/kg body weight and day)	3.7 mg/kg body weight: NOAEL ≥ 38 mg/kg body weight: slight vacuolation of Sertoli cells ≥ 375 mg/kg body weight: severe vacuolation of Sertoli cells, testicular atrophy	Poon <i>et al.</i> 1997 (see Table 2)
rat, SD, groups of 15 ♂, age not specified	17 weeks, 0, 2000, 10000, 20000 mg/kg diet (0, 143, 737, 1440 mg/kg body weight and day)	143 mg/kg body weight: castration cells in the pituitary gland, slight testicular changes (not significant) ≥ 737 mg/kg body weight: absolute and relative testis weights decreased, testicular atrophy	Gray <i>et al.</i> 1977 (see Table 2)
rat, “albino”, groups of 6 ♂, no other details	15 days, 0, 500, 1000 mg/kg body weight and day, p.o.	500 mg/kg body weight: sperm count in the epididymis decreased 1000 mg/kg body weight: absolute and relative epididymis weights decreased, sperm count in the epididymis decreased, enzymes of spermatogenesis changed	Siddiqui and Srivastava 1992

PND: postnatal day

Effects on the testes in the form of slight testicular atrophy were reported in adult **F344 rats** given gavage doses of 1000 mg/kg body weight and day or more for 14 days (NOAEL 330 mg/kg body weight; Agarwal *et al.* 1986a; Table 3). Testis weights were reduced after DEHP doses of 5000 mg/kg diet (284 mg/kg body weight and day) or more for 60 days (NOAEL 69 mg/kg body weight; Agarwal *et al.* 1986b; Table 3). After administration of doses of 500 mg/kg diet (30 mg/kg body weight and day) for 104 weeks there was an increase in the incidence of bilateral aspermatogenesis which occurs in ageing rats (David *et al.* 2000a). Degeneration of the seminiferous tubules (NTP 1982) and reduced testis weights (David *et al.* 2000a) were observed after administration of the highest doses of 12000 mg/kg diet (670 mg/kg body weight and day, and 850 mg/kg body weight and day) for 103 and 104 weeks (see Tables 2, 3). **No effects on the testes were seen in the 104-week study after doses of 100 mg/kg diet (6 mg/kg body weight and day; David *et al.* 2000a).**

In a mechanistic study of DEHP, 3-day-old male **Sprague-Dawley rats** were given single gavage doses of the substance. After doses of 100 mg/kg body weight or more, proliferation in Sertoli cells and cyclin D2 expression were decreased. After doses of 200 mg/kg body weight or more, multinuclear gonocytes were found. No effects were observed at 20 mg/kg body weight (Li *et al.* 2000; Table 3). After administration of doses of 1000 mg/kg body weight and day for 5 days, effects on Sertoli cells were observed in 1-week-old male Sprague-Dawley rats, and effects on the spermatocytes in animals from the age of 2 weeks and, in addition, the loss of spermatids in animals of 6 and 12 weeks. Mating of the animals exposed at the age of 1 week did not reveal impairment in fertility, however (Dostal *et al.* 1988). In another study, after administration of DEHP in the diet for 6 weeks, effects on the testes were observed only after doses of 1500 mg/kg body weight or more. These could be avoided by the administration of vitamin C or E (Ishihara *et al.* 2000; Table 3). Gavage doses of 2000 mg/kg body weight and day for up to 14 days caused a depletion in the level of zinc in the testes, which was found to be a secondary effect of the testicular toxicity (Park *et al.* 2002). In Sprague-Dawley rats given doses of 500 mg/kg diet (38 mg/kg body weight and day) or more for 13 weeks, slight vacuolation of Sertoli cells was observed, and after doses of 5000 mg/kg diet (375 mg/kg body weight and day) or more also tubular atrophy in the testes. **The NOAEL was given as 50 mg/kg diet (3.7 mg/kg body weight and day)** (Poon *et al.* 1997; Tables 2 and 3). In a 3-generation study with Sprague-Dawley rats, which, however, is only available as an abstract and poster presentation, increased liver weights and histological changes in the liver, and increased kidney weights and decreased weights of the accessory sexual organs were observed in the male animals after doses of 1000 mg/kg diet (80 mg/kg body weight and day) or more. After doses of 7500 mg/kg diet (600 mg/kg body weight and day) or more, also histological changes in the testes, epididymis, kidneys and adrenal gland were detected. At this dose there were also effects on the offspring (reduced litter size, reduced number of male pups, reduced body weight gains in the pups, testicular atrophy, reduced anogenital distance, delayed sexual development, reduced number of spermatids). The authors conclude that up to doses of 1000 mg/kg diet (80 mg/kg body weight and day) no general toxic effects on reproduction, apart from liver toxicity (histopathological changes), are to be observed (Wolfe *et al.* 2001; Table 4). At this dose, however, there

were still changes in organ weights, including the male accessory sexual organs. **No dose-dependent effects were found in this study at doses of 300 mg/kg diet (24 mg/kg body weight and day).** Merely the testes and epididymis were aplastic in one male F₁ offspring from this dose group and the seminal vesicles were small in two of the male F₁ offspring. As such effects were not found in the offspring of the next highest dose group, the authors conclude that the findings must be verified in repeat experiments before they are used as the basis for evaluations. In an earlier 17-week study with Sprague-Dawley rats, after doses of 2000 mg/kg diet (143 mg/kg body weight and day), slight effects on the testes were reported which were not significant; these effects were found to be significant at the next highest dose (Gray *et al.* 1977; Tables 2 and 3).

Table 4. Fertility studies with DEHP

Species, strain, number of animals	Exposure	Findings	References
rat, Wistar, groups of 25 ♂, 25 ♀	2 generations, 0, 1000, 3000, 9000 mg/kg diet (about 0, 113, 340, 1088 mg/kg body weight and day)	<p>≥ 113 mg/kg body weight: parent animals: relative liver weights increased (F₁: ♀); offspring: liver weights increased (F₁, F₂)</p> <p>≥ 340 mg/kg body weight: parent animals: NOAEL (fertility), absolute and relative liver weights increased (F₀), hypertrophy (F₀) and eosinophilia (F₀, F₁) of the liver; offspring: number of stillbirths increased (F₂), survival decreased (F₁, F₂), body weight gains decreased (F₂), anogenital distance decreased (F₁, F₂: ♂), thymus weights decreased (F₁, F₂: ♂), number of offspring with rudimentary areolas/nipples increased (F₂: ♂)</p> <p>1088 mg/kg body weight: parent animals: mortality increased (F₀, F₁: ♀), food consumption decreased (F₀: ♀; F₁: ♀, ♂), body weight gains decreased (F₀: ♀; F₁: ♂, ♀), fertility indices decreased (F₀, F₁: ♂, ♀), implantations decreased (F₁: ♀), postimplantation losses increased (F₀, F₁), no sperms (F₀: 1/25 ♂), sperm count decreased (F₀, F₁: ♂), tubular atrophy in the testes (F₀, F₁: 5/25 and 6/25 ♂), aspermia (F₀, F₁: in both groups 2/25 ♂), number of abnormal sperms increased (F₁: ♂), foci in clear cells of the liver increased (F₀, F₁), number of follicles and <i>corpora lutea</i> decreased (F₀, F₁: ♀), visible malformations of the sexual organs (F₁: 2/25 ♂), chronic progressive nephropathy (F₁); offspring: litter size decreased (F₁), number of stillbirths increased (F₁), body weight gains decreased (F₁), number of young with rudimentary areolas/nipples increased (F₁: ♂), delayed opening of the vagina (F₁: ♀) and development of the prepuce (F₁: ♂), spleen weights decreased (F₁, F₂: ♂), testis weights decreased (F₂: ♂)</p>	BASF 2001

Table 4. continued

Species, strain, number of animals	Exposure	Findings	References
rat, Sprague-Dawley , groups of 17 ♂, 17 ♀	3 generations (F _{1a} , 1b, 1c, F _{2a} , 2b, 2c, F _{3a} , 3b, 3c), 1.5 (controls), 10, 30, 100, 300, 1000, 7500, 10000 mg/kg diet (about 0.12, 0.8, 2.4, 8, 24, 80, 600, 800 mg/kg body weight; estimated according to Poon <i>et al.</i> 1997)	24 mg/kg body weight: <u>parent animals</u> : no effects; <u>offspring</u> (F ₁ : 1–2 ♂): aplastic testes and epididymis, small seminal vesicles (not at next highest dose) ≥ 80 mg/kg body weight: <u>parent animals</u> : liver weights increased, histological changes in the liver, kidney weights increased, weights of male accessory sexual organs decreased; <u>offspring</u> : no effects ≥ 600 mg/kg body weight: <u>parent animals</u> : body weight gains decreased, pregnancy indices decreased, histological changes in the testes, epididymis, adrenal gland, kidneys; <u>offspring</u> : litter size decreased, number of male animals/litter decreased, body weight gains decreased (♂, ♀), testicular atrophy (♂), anogenital distance decreased (♂), delayed sexual development (♂, ♀), number of spermatids decreased (♂) 800 mg/kg body weight: <u>parent animals</u> : number of spermatids decreased, no F ₂ offspring	Wolfe <i>et al.</i> 2001 (abstract, poster)
mouse, CD-1 , groups of 20 ♂, 20 ♀	continuous mating , exposure for 7 days before mating, 98 days during cohabitation and 21 days afterwards; 0, 100, 1000, 3000 mg/kg diet (about 20, 200, 600 mg/kg body weight and day)	20 mg/kg body weight and day: NOAEL (fertility) ≥ 200 mg/kg body weight and day: fertility decreased (♂, ♀), number of litters/pair decreased, number of live offspring decreased 600 mg/kg body weight and day ♂: fertility decreased, testis and epididymis weights decreased, prostate weights decreased, sperm motility decreased, number of abnormal sperms increased, atrophy of the seminiferous tubules; ♀: infertile, liver weights increased, pituitary weights decreased, ovary weights decreased	Lamb <i>et al.</i> 1987

In a study with continuous mating, adult **CD-1 mice** were exposed to DEHP for 7 days before mating, 98 days during cohabitation and 21 days afterwards. Fertility was reduced after doses of 1000 mg/kg diet (about 200 mg/kg body weight and day) or more. At 3000 mg/kg diet (about 600 mg/kg body weight and day) also testis, epididymis and prostate weights were reduced, sperm motility was decreased, the number of abnormal sperms was increased and the seminiferous tubules were atrophic. The female animals were infertile and the pituitary and ovary weights reduced. **No effects were found at doses of 100 mg/kg diet (20 mg/kg body weight and day)** (Lamb *et al.* 1987; Table 4).

In a 104-week study with **B6C3F₁ mice**, testis weights were reduced after doses of 500 mg/kg diet (about 100 mg/kg body weight and day) or more. After doses of 1500 mg/kg diet (about 300 mg/kg body weight and day) and above, bilateral

hypospermia was observed in the testes and immature or abnormal sperms were found in the epididymis. **No effects were found at doses of 100 mg/kg diet (about 20 mg/kg body weight and day)** (David *et al.* 1999, 2000b; Table 2).

Studies with other administration routes (intravenous or intraperitoneal) are not considered here (see ECB 2000, IARC 2000, WHO 1992).

5.5.2 Developmental toxicity

DEHP is transferred through the placenta to the offspring. In lactating rats, DEHP is extracted efficiently from the serum into the milk of the dams. After high doses of DEHP (2000 mg/kg body weight and day), also the quality and quantity of the milk changes (Dostal *et al.* 1987a).

Studies of developmental toxicity can be found in Table 5.

5.5.2.1 Inhalation

In a study of developmental toxicity with inhalation exposure of **Wistar rats** (head/nose only) to concentrations of DEHP of 10, 50 or 300 mg/m³ on days 6 to 15 of gestation, at the highest concentration there was a significant increase in the incidence of litters with retarded development (controls 16.7 %; low concentration 33.3 %; middle concentration 31.3 %; high concentration 56.3 %, $p = 0.05$). Dilatation of the renal pelvis was found most often. According to the authors, this is a very frequent finding in this strain of rat, and is therefore not regarded as exposure-related. Maternal toxicity was manifest in reduced body weight gains on day 21 *post partum* in the high exposure group (Merkle *et al.* 1988). **The NOAEL in this study was 50 mg/m³ (estimated to be about 18 mg/kg body weight and day)** for maternal and developmental toxicity.

5.5.2.2 Ingestion

In a study of developmental toxicity with **Wistar rats** given gavage doses of DEHP on days 6 to 15 of gestation, **the NOAEL for maternal and developmental toxicity was given as 200 mg/kg body weight and day**. Doses of 1000 mg/kg body weight and day caused maternal toxicity and developmental toxicity (increased foetal mortality), including malformations (tail, brain, urinary tract, gonads, sternum) (Hellwig *et al.* 1997). Also intraperitoneal administration of single doses of 12.5 or 25 mmol/l (about 4900 or 9800 mg/kg body weight and day) caused an increase in malformations (kidneys, cardiovascular system, tail) (Ritter *et al.* 1987). In a 2-generation study with Wistar rats (prenatal and postnatal exposure of the F₁ generation), in male and female offspring effects were seen on the liver (organ weight increases) after the low dose (corresponding to 113 mg/kg body weight and day) and doses above this. Toxic effects (mortality, reduced body weight gains) and delays in the sexual development of the male offspring (decreased anogenital distance in F₁ and F₂ animals, an increase in rudimentary areolas/nipples in F₂ animals) were not observed until the next highest dose (corresponding to 340 mg/kg body weight and day) (see Section 5.5.1.2; BASF 2001).

Table 5. Studies of developmental toxicity (exposure of the dams)

Species, strain, number of animals	Exposure	Findings	References
rat, Wistar, groups of 25 ♀	GD 6–15 , 0, 10, 50, 300 mg/m ³ , 6 h/day, examination on GD 20 (n = 20) and PND 21 (n = 5)	50 mg/m³: NOAEL (maternal toxicity, developmental toxicity) (implantation losses increased only at this concentration) 300 mg/m³: dams: body weight gains decreased (PND 21); foetuses: retardation (dilatation of the renal pelvis) increased (high incidence in historical controls; no other details); offspring: no impairment of postnatal development up to PND 21	Merkle <i>et al.</i> 1988
rat, Wistar, groups of 10 ♀	GD 6–15 , 0, 40, 200, 1000 mg/kg body weight, p.o., examination on GD 20	200 mg/kg body weight: NOAEL (maternal toxicity, developmental toxicity) 1000 mg/kg body weight: dams: food consumption decreased, body weight gains decreased, relative kidney weights increased, relative liver weights increased; foetuses: postimplantation losses increased, number of live foetuses decreased, malformations (e.g. tail, brain, urinary tract, gonads, sternum)	Hellwig <i>et al.</i> 1997
rat, Wistar, ♀ (number of animals not specified)	GD 12 , 12.5, 25 mmol/l, intraperitoneal (about 4900, 9800 mg/kg body weight), examination on GD 20	4900 mg/kg body weight: foetuses: malformations (4.5 %) 9800 mg/kg body weight: foetuses: malformations (21 %; e.g. hydronephrosis, cardiovascular malformations, tail malformations)	Ritter <i>et al.</i> 1987
rat, F344, groups of 16–21 ♀	GD 6–19 , a) 0, 1125, 1500 mg/kg body weight and day, p.o. b) 0, 333, 500, 750, 1125 mg/kg body weight and day, p.o., examination up to PND 6	≥ 750 mg/kg body weight: offspring: malformations (e.g. microphthalmia, cleft palates) ≥ 1125 mg/kg body weight: dams: body weight gains decreased (GD 6–8), resorptions increased; offspring: mortality	a) Narotsky and Kavlock 1995, b) Narotsky <i>et al.</i> 1995
rat, F344, groups of 22–25 ♀	GD 0–20 , 0, 5000, 10000, 15000, 20000 mg/kg diet (357, 666, 856, 1055 mg/kg body weight and day), examination on GD 21	≥ 357 mg/kg body weight: dams: food consumption decreased, water consumption increased, liver weights increased; foetuses: NOAEL (developmental toxicity) 666 mg/kg body weight: dams: body weight gains decreased; foetuses: body weights decreased 1055 mg/kg body weight: foetuses: resorptions increased, number of live foetuses decreased	Tyl <i>et al.</i> 1988

Table 5. continued

Species, strain, number of animals	Exposure	Findings	References
rat, F344, ♀	GD 0–20, 0, 2500, 5000, 10000 mg/kg diet (164, 313, 573 mg/kg body weight and day), examination over 2 generations up to PND 128	164 mg/kg body weight: NOAEL (maternal toxicity, developmental toxicity) ≥ 313 mg/kg body weight: <u>dams</u> : food consumption decreased, body weight gains decreased; <u>offspring</u> : birth weights decreased, body weight gains decreased, survival rate decreased	NTP 1986
rat, SD, groups of 6–8 ♀	GD 3 to LD 21, 375, 750, 1500 mg/kg body weight, p.o., examination of offspring PND 1–105	≥ 375 mg/kg body weight: <u>offspring</u> ♂: formation of nipples (PND 14), testis weights decreased (PND 21) ≥ 750 mg/kg body weight: <u>offspring</u> ♂: survival decreased, anogenital distance decreased (PND 1), effects on prostate, seminal vesicles (PND 21), sperm count in epididymis decreased (PND 63) 1000 mg/kg body weight: <u>offspring</u> ♂: incomplete development of the prepuce; ♀: body weight gains decreased	Moore <i>et al.</i> 2001
rat, SD, groups of 2–4 ♀	GD 14 to LD 3, 750 mg/kg body weight and day, p.o., examination of the offspring on GD 17, 18, 20 and PND 2	750 mg/kg body weight: <u>dams</u> : body weight gains decreased; <u>offspring</u> ♂: testosterone production decreased (GD 17, 18 and 20 and PND 2), foetal testis weights decreased, Leydig cell hyperplasia, multinuclear gonocytes (GD 20, PND 2), anogenital distance decreased (PND 2)	Parks <i>et al.</i> 2000
rat, SD, 16 ♀	GD 14 to LD 3, 750 mg/kg body weight and day, p.o., examination of the offspring from PND 1 to the age of 7 months	750 mg/kg body weight: <u>dams</u> : body weight gains decreased; <u>offspring</u> ♂, ♀: birth weights decreased; ♂: anogenital distance decreased, testis weights decreased, multinuclear gonocytes (♂, PND 2–3), formation of nipples (PND 9–10), delayed preputial opening as a result of genital malformations (testes, prostate gland, seminal vesicles, sex accessory glands)	Gray <i>et al.</i> 2000
rat, Long Evans, groups of at least 9 ♂ offspring	GD 12–21, 100 mg/kg body weight and day, p.o., examination of the offspring (♂) PND 21, 35 and 90	100 mg/kg body weight: <u>offspring</u> ♂: serum testosterone and luteinizing hormone decreased (PND 21, PND 35, no longer by PND 90); no effects on testis weights	Akingbemi <i>et al.</i> 2001

Table 5. continued

Species, strain, number of animals	Exposure	Findings	References
rat, Long Evans , groups of 7 ♀	LD 1–21 , 100 mg/kg body weight and day, p.o., examination of the offspring PND 21, 35 and 90	100 mg/kg body weight: offspring ♂ : serum testosterone (but not serum luteinizing hormone) slightly decreased (PND 21, but not PND 35, 90); no effects on testis weights	Akingbemi <i>et al.</i> 2001
rat, Long-Evans , groups of 12 ♀	GD 1 to LD 21 , 32.5, 325 µl/l drinking water (about 3.5, 35 mg/kg body weight and day), examination of the offspring PND 21, 28, 35, 42 and 56	≥ 3.5 mg/kg body weight: dams : 0.20 ± 0.03 µg/ml plasma (LD 21); offspring : DEHP not detectable in serum; ♂: absolute testis weights decreased; absolute kidney weights decreased, glomerulonephritis, dilatation of the renal pelvis, slight fibrosis in the kidneys, subendothelial oedema in the liver (PND 21) 35 mg/kg body weight: dams : 1.42 ± 0.21 µg/ml plasma (LD 21); offspring : 0.50 ± 0.06 µg/ml (PND 21); ♂, ♀: body weight gains decreased (PND 56); ♀: more time taken in a behavioural test (beam walking test)	Arcadi <i>et al.</i> 1998
rat, “albino”, 4 ♀	LD 1–21 , 2000 mg/kg body weight, p.o., examination of the offspring PND 31, 61 and 91	2000 mg/kg body weight: offspring ♂ : absolute testis weights decreased (PND 31), body weight gains decreased (PND 61 and 91), sperm count in epididymis decreased (PND 91); testicular enzymes changed (γ-GT, SDH, LDH, β-glucuronidase, acid phosphatase), testosterone concentration unchanged	Tandon <i>et al.</i> 1990
mouse, CD-1 , groups of 24–30 ♀	GD 0–17 , 0, 250, 500, 1000, 1500 mg/kg diet (0, 44, 91, 191, 292 mg/kg body weight and day), examination on GD 17	44 mg/kg body weight: NOAEL (developmental toxicity) 91 mg/kg body weight: NOAEL (maternal toxicity); foetuses : malformations increased (e.g. open eyes, exophthalmia, exencephaly, tail short or missing, changes in the main blood vessels, ribs fused or branched, thoracic vertebrae fused) 191 mg/kg body weight: dams : body weight gains decreased, ovary weights decreased, relative liver weights decreased, resorptions increased; foetuses : late foetal mortality, number of live foetuses decreased, foetal body weights decreased 292 mg/kg body weight: dams : food and water consumption increased	Tyl <i>et al.</i> 1988

Table 5. continued

Species, strain, number of animals	Exposure	Findings	References
mouse, CD-1, ♀	GD 0–17, 0, 100, 250, 500 mg/kg diet (0, 19, 48, 95 mg/kg body weight and day), examination over 2 generations up to PND 169	48 mg/kg body weight: NOAEL (maternal toxicity, developmental toxicity) 95 mg/kg body weight: dams: minimal toxicity (body weight gains decreased on LD 4 and 7); offspring: number of live offspring decreased, survival (PND 1–4) decreased; (no changes: postnatal development, incisor eruption, grasping reflex, eye opening, descent of the testes, vaginal opening, spontaneous locomotor activity)	NTP 1988
mouse, ICR-JCL, groups of 7–12 ♀	GD 1–17, 0, 500, 1000, 2000, 4000, 10000 mg/kg diet (0, 70, 190, 400, 830, 2200 mg/kg body weight and day), examination on GD 18	70 mg/kg body weight: foetuses: delayed ossification (not in the next highest dose group) ≥ 190 mg/kg body weight: foetuses: resorptions increased, dead foetuses increased, deficient ossification of the sternbrae, lumbar ribs increased 400 mg/kg body weight: dams: body weight gains decreased; offspring: foetal body weights decreased, malformations (exencephaly, spina bifida, tail malformations, club feet) 830, 2200 mg/kg body weight: no live offspring	Shiota <i>et al.</i> 1980
mouse, ICR, groups of 7–24 ♀	GD 1–17, 0, 500, 1000, 2000, 4000, 10000 mg/kg diet (0, 75, 150, 300, 600, 1500 mg/kg body weight and day), examination on GD 18	75 mg/kg body weight: NOAEL (developmental toxicity) 300 mg/kg body weight: NOAEL (maternal toxicity); foetuses: number of live foetuses decreased, embryotoxicity, malformations ≥ 600 mg/kg body weight: dams: body weight gains decreased; foetuses: malformations (e.g. exencephaly, tail anomalies)	Shiota and Nishimura 1982
mouse, ICR, groups of 9–11 ♀	GD 7–9, 0, 250, 500, 1000, 2000 mg/kg body weight and day, p.o., examination on GD 18	500 mg/kg body weight: NOAEL (developmental toxicity) ≥ 1000 mg/kg body weight, p.o.: foetuses: resorptions increased, foetal body weights decreased, malformations (exencephaly, anencephaly, open eyelids, tail anomalies)	Shiota and Mima 1985
mouse, ICR, groups of 3–9 ♀	GD 7–9, 0, 500, 1000, 2000, 4000, 8000 mg/kg body weight and day, intraperitoneal, examination on GD 18	4000 mg/kg body weight: NOAEL (developmental toxicity) 8000 mg/kg body weight, intraperitoneal: foetuses: resorptions increased, foetal body weights decreased, malformations (exencephaly, anencephaly, open eyelids, tail anomalies)	Shiota and Mima 1985

Table 5. continued

Species, strain, number of animals	Exposure	Findings	References
mouse, ddY-SlcxCBA , groups of at least 3 ♀	single doses on GD 6, 7, 8, 9 or 10 , 0, 0.05, 0.1, 1.0, 7.5, 10, 30 ml/kg body weight, p.o. (about 0, 50, 100, 1000, 7500, 10000, 30000 mg/kg body weight and day) examination on GD 18	50 mg/kg body weight: foetal body weights decreased (GD 7) ≥ 100 mg/kg body weight: <u>foetuses</u> : number of live foetuses decreased (GD 7) ≥ 1000 mg/kg body weight: <u>foetuses</u> : malformations increased (in particular exencephaly, open eyelids, tail malformations, club feet, skeletal anomalies)	Tomita <i>et al.</i> 1982a, Yagi <i>et al.</i> 1980
mouse, PPARα^{+/+} (C57BL/N6x Sv/129), PPARα^{-/-} , groups of 10 ♀	GD 8–9 , 1000 mg/kg body weight, p.o., examinations on GD 10 and 18	1000 mg/kg body weight: <u>dams</u> : body weight gains decreased, relative liver weights increased; <u>foetuses</u> : resorptions, foetal body weights decreased, anomalies increased; (no differences between PPARα ^{+/+} and PPARα ^{-/-} mice)	Peters <i>et al.</i> 1997

GD gestation day, LD lactation day, PND post-natal day

In **F344** rats given doses of DEHP of more than 1000 mg/kg body weight and day on days 6 to 19 of gestation, reduced body weight gains were reported in the dams, total resorptions, mortality, and malformations in the offspring (Narotsky and Kavlock 1995, Narotsky *et al.* 1995). When F344 rats were given DEHP with the diet on days 0 to 20 of gestation, maternal toxicity was observed after doses of 5000 mg/kg diet (357 mg/kg body weight and day) or more. In the foetuses body weights were reduced after doses of 10000 mg/kg diet (666 mg/kg body weight and day) or more, and at 15000 mg/kg diet mortality was increased (Tyl *et al.* 1988). When the offspring were observed for 128 days after birth, body weight gains and survival were reduced after doses of 5000 mg/kg diet (313 mg/kg body weight and day) or more. The NOAEL (maternal toxicity, developmental toxicity) in this study was 2500 mg/kg diet (164 mg/kg body weight and day) (NTP 1986).

When **Sprague-Dawley** rats were exposed from day 3 of gestation to the end of lactation, in the male offspring there was an increase in the number of pups with areola and nipple retention (14 days after birth) and reduced testis weights (21 days after birth) after the lowest dose (375 mg/kg body weight and day) and doses above this. In the offspring of the middle dose group (750 mg/kg body weight and day) survival was reduced, the anogenital distance decreased (1 day after birth), and effects on the prostate gland and seminal vesicle were observed (21 days after birth). In young adult animals (63 days after birth) the sperm count in the epididymis was reduced. Effects (reduced body weight gains) were observed in the female offspring only at the high dose (1000 mg/kg body weight and day) (Moore *et al.* 2001). The administration of doses of DEHP of 750 mg/kg body weight to Sprague-Dawley rats from day 14 of gestation to a

DEHP of 750 mg/kg body weight to Sprague-Dawley rats from day 14 of gestation to a maximum of day 3 of lactation (Gray *et al.* 2000, Parks *et al.* 2000) led to reduced body weight gains in the dams. In the male offspring sexual differentiation was impaired. In male foetuses (day 20 of gestation) and newborn male pups (2 days after birth) testosterone production and testis weights were decreased, hyperplasia of Leydig cells and multinuclear gonocytes were observed, and in the newborn the anogenital distance was reduced (Parks *et al.* 2000). In 9 to 10-day-old pups, an increase in the number of animals with rudimentary areolas/nipples was observed and the development of the prepuce was delayed as a result of genital malformations (Gray *et al.* 2000).

In a drinking-water study in which **Long-Evans rats** were given DEHP in the drinking-water from day 1 of gestation to the end of lactation, even in the male offspring of the low dose group (32.5 µl/l drinking-water, corresponding to about 3.5 mg/kg body weight and day), effects on the testes (reduced absolute testis weights), kidneys (reduced absolute kidney weights, nephritis, slight fibrosis) and liver (oedema) were observed 21 days after birth. As DEHP is practically insoluble in water, the substance was distributed in the drinking-water by ultrasonic waves. Determinations of DEHP in the plasma of dams and pups confirm the uptake of DEHP (Arcadi *et al.* 1998). In a study with Long-Evans rats, however, with gavage administration of DEHP of 100 mg/kg body weight and day on days 12 to 21 of gestation or 1 to 21 of lactation, reduced testosterone concentrations were found, but no effects on testis weights (Akingbemi *et al.* 2001). As a result of the prenatal and postnatal exposure of the pups, this study is not relevant for the evaluation of a threshold concentration at the workplace.

When **rats** (strain not specified) were given doses of DEHP of 2000 mg/kg body weight and day during lactation, impairment in the maturation of the testes and a loss of germ cells in early stages were observed in the male offspring (Tandon *et al.* 1990).

In a developmental toxicity study with **CD-1 mice** with administration of DEHP with the diet to pregnant animals on days 0 to 17 of gestation, the number of malformed foetuses was increased after doses of 91 mg/kg body weight or more. Maternal toxicity was observed after doses of 191 mg/kg body weight or more. **The NOAEL for developmental toxicity was 44 mg/kg body weight, the NOAEL for maternal toxicity 91 mg/kg body weight and day** (Tyl *et al.* 1988). In another study with CD-1 mice, after administration of DEHP on days 0 to 17 of gestation, **the NOAEL (maternal toxicity, developmental toxicity) was given as 48 mg/kg body weight and day**. Postnatal survival was decreased at the next highest dose of 95 mg/kg body weight and day, but no other changes in postnatal development were observed. Incisor eruption, the grasping reflex, the opening of the eyes, the descent of the testes, the opening of the vagina and spontaneous locomotor activity were investigated (NTP 1988).

When DEHP was given to **IRC mice** on days 1 to 17 of gestation (Shiota and Nishimura 1982, Shiota *et al.* 1980), increased mortality and delayed ossification of the sternebrae were observed in the foetuses after doses of 1000 mg/kg diet (190 mg/kg body weight and day) or more, malformations (exencephaly, spina bifida, tail malformations, club feet) after 2000 mg/kg diet (400 mg/kg body weight and day) or more (Shiota *et al.* 1980). **The NOAEL for developmental toxicity was 75 mg/kg body weight and day, the NOAEL for maternal toxicity 300 mg/kg body weight and day** (Shiota and Nishimura 1982). After gavage administration of DEHP on days 7 to 9

of gestation, the NOAEL for developmental toxicity was 500 mg/kg body weight and day, after intraperitoneal administration 4000 mg/kg body weight and day (Shiota and Mima 1985).

ddY-SlcxCBA mice were given single doses of DEHP on days 6, 7, 8, 9 or 10 of gestation; with exposure on day 7 of gestation reduced foetal body weights were observed at doses of 50 mg/kg body weight, increased foetal mortality after doses of 100 mg/kg body weight or more and malformations (in particular exencephaly, open eyelids, tail malformations, club feet, skeletal anomalies) after doses of 1000 mg/kg body weight or more (Tomita *et al.* 1982a, Yagi *et al.* 1980).

In a mechanistic study of DEHP, **PPAR α ^{+/+}** (C57BL/N6×Sv/129) **and PPAR α ^{-/-} mice** were given doses of 1000 mg/kg body weight on days 8 and 9 of gestation. Irrespective of the expression of PPAR α , maternal toxicity (e.g. reduced body weight gains and increased liver weights) and foetal toxicity (e.g. resorptions, reduced body weights, anomalies) were observed (Peters *et al.* 1997). The authors conclude that the developmental toxicity of DEHP is not PPAR α -mediated.

5.6 Genotoxicity

The genotoxic effects of DEHP and its main metabolite MEHP have been tested in numerous test systems.

5.6.1 *In vitro*

The *in vitro* studies of the genotoxicity of DEHP are summarized in Table 6.

5.6.1.1 Bacteria

In the bacterial mutagenicity tests with *Salmonella typhimurium* and *Escherichia coli*, **DEHP** was not found to have mutagenic effects in either the presence or absence of a metabolic activation system (see Table 6).

5.6.1.2 Yeasts/Fungi

In most of the studies with **DEHP** and *Saccharomyces cerevisiae*, neither gene mutation (Arni 1985, Inge-Vechtomov *et al.* 1985, Parry and Eckardt 1985) nor recombination (Arni 1985, Brooks *et al.* 1985, Carls and Schiestl 1994, Inge-Vechtomov *et al.* 1985, Parry and Eckardt 1985) was observed. In one study (Mehta and von Borstel 1985) DEHP increased the frequency of recombinations (gene conversions) and point mutations at pH 6.3, while at pH 7.0 these effects did not occur or were not reproducible. The reason for this pH-dependency is unclear. The increased incidence of aneuploidy observed in a study was regarded by the authors themselves as questionable (Parry and Eckardt 1985).

In *Saccharomyces pombe* DEHP did not cause reproducible increases in the mutation frequency in either the presence or the absence of S9 mix (Loprieno *et al.* 1985).

In *Aspergillus nidulans* DEHP did not induce either mutation, recombination or nondisjunction (Carere *et al.* 1985).

5.6.1.3 Mammalian cells

DEHP did not induce sister chromatid exchange (SCE) in CHO cells (a cell line derived from Chinese hamster ovary) (Abe and Sasaki 1977, Douglas *et al.* 1985, Gulati *et al.* 1985), in the rat liver cell line RL4 (Priston and Dean 1985) and in human lymphocytes (Obe *et al.* 1985). A very slight, but statistically significant increase in the incidence of SCE caused by DEHP was observed after co-cultivation of human lymphocytes with rat hepatocytes (Lindahl-Kiessling *et al.* 1989).

DNA strand breaks were not caused by DEHP in either primary hepatocytes of the rat (Bradley 1985, Schmezer *et al.* 1988) or of the hamster (Schmezer *et al.* 1988), or in CHO cells (Douglas *et al.* 1985). In human lymphocytes DEHP induced strand breaks in the absence of S9 mix, while in human leukocytes the induction of reproducible strand breaks was not detected either in the presence or in the absence of S9 mix (Anderson *et al.* 1999b). In primary hepatocytes of the rat, there was no evidence of covalent binding of DEHP or its metabolites with DNA (Gupta *et al.* 1985). In UDS tests with cultured hepatocytes of the mouse (Smith-Oliver and Butterworth 1987), the rat (Astill *et al.* 1986, Butterworth *et al.* 1984, Kornbrust *et al.* 1984, Probst and Hill 1985, Williams *et al.* 1985) and humans (Butterworth *et al.* 1984) DEHP did not induce repair synthesis.

In several TK^{+/-} mutation tests with L5178Y mouse lymphoma cells (Amacher and Turner 1985, Astill *et al.* 1986, Kirby *et al.* 1983, Myhr *et al.* 1985, Styles *et al.* 1985) and human TK6 cells (Crespi *et al.* 1985), DEHP was not found to be mutagenic. In the Na⁺/K⁺-ATPase gene mutation test DEHP did not induce gene mutation in either L5178Y mouse lymphoma cells (Garner and Campbell 1985, Styles *et al.* 1985) or in BALB/c-3T3 cells (Matthews *et al.* 1985). Also in an HPRT gene mutation test with the human lymphoblast cell line AHH-1 (Crespi *et al.* 1985) DEHP was not found to have mutagenic activity. Most of the mutation tests named above were carried out both in the presence and in the absence of a metabolic activation system.

DEHP was not found to have clastogenic properties in numerous chromosomal aberration tests with hamster cells, such as CHO cells (Abe and Sasaki 1977, Gulati *et al.* 1985, Phillips *et al.* 1982), CHL cells (a cell line derived from Chinese hamster liver) (Ishidate and Odashima 1977, Ishidate and Sofuni 1985), CH1-L cells (primary liver cells of the hamster) (Danford 1985), or with RL4 rat liver cells (Priston and Dean 1985) and human lymphocytes (Stenchever *et al.* 1976, Tsuchiya and Hattori 1979, Turner *et al.* 1974). In SHE cells (primary cells from Syrian hamster embryos), however, DEHP increased the incidence of structural chromosomal aberrations in the presence of S9 mix in a concentration-dependent manner (Tsutsui *et al.* 1993).

Micronucleus tests with CHO cells (Douglas *et al.* 1985) and primary rat hepatocytes (Müller-Tegethoff *et al.* 1995) yielded negative results. DEHP was, however, observed to induce micronuclei in SHE cells (Fritzenschaf *et al.* 1993).

Table 6. *In vitro* studies of genotoxicity

Test system		Concentration ^a	Results ^b		References
			–S9	+S9	
DEHP					
Bacteria					
rec assay	<i>Bacillus subtilis</i>	500 µg/filter	–		Tomita <i>et al.</i> 1982b
BMT	<i>Salmonella typhimurium</i> TA1537, TA98, TA7001, TA7002, TA7003, TA7004, TA7005, TA7006	1000 µg/ml	–	–	Gee <i>et al.</i> 1998
BMT	<i>S. typhimurium</i> TA100, TA98	2000 µg/plate	–	–	Yoshikawa <i>et al.</i> 1983
BMT	<i>S. typhimurium</i> TA100, TA98	4000 µg/plate	–	–	Robertson <i>et al.</i> 1983
BMT	<i>S. typhimurium</i> TA100, TA102, TA98, TA97	5000 µg/plate	–	–	Matsushima <i>et al.</i> 1985
BMT	<i>S. typhimurium</i> TA100, TA1535, TA1537, TA1538, TA98	5000 µg/plate	–	–	Rexroat and Probst 1985
BMT	<i>S. typhimurium</i> TA100, TA1535, TA1537, TA1538, TA98	5000 µg/plate	–	–	Simmon <i>et al.</i> 1977
BMT	<i>S. typhimurium</i> TA100	5000 µg/plate	n.d.	(+)	Tomita <i>et al.</i> 1982b
BMT	<i>S. typhimurium</i> TA100, TA1535, TA1537, TA1538, TA98	9860 µg/plate	–	–	Kirby <i>et al.</i> 1983
BMT	<i>S. typhimurium</i> TA100, TA102, TA98, TA97	10000 µg/plate	–	–	Baker and Bonin 1985
BMT	<i>S. typhimurium</i> TA100, TA102, TA98, TA97	10000 µg/plate	–	–	Nohmi <i>et al.</i> 1985
BMT	<i>S. typhimurium</i> TA100, TA1535, TA98, TA97	10000 µg/plate	–	–	Zeiger and Haworth 1985
BMT	<i>S. typhimurium</i> TA100, TA1535, TA98, TA1537	10000 µg/plate	–	–	Zeiger <i>et al.</i> 1985
BMT	<i>Escherichia coli</i> WP2 uvrA	2000 µg/plate	–	–	Yoshikawa <i>et al.</i> 1983
Yeasts/fungi					
mutation	<i>Saccharomyces cerevisiae</i>	1000 µg/ml	–	–	Inge-Vechtomov <i>et al.</i> 1985
mutation	<i>Saccharomyces cerevisiae</i>	5000 µg/ml	–	–	Arni 1985

Table 6. continued

Test system		Concentration ^a	Results ^b		References
			-S9	+S9	
mutation	<i>Saccharomyces cerevisiae</i>	5000 µg/ml	-	-	Parry and Eckardt 1985
mutation	<i>Saccharomyces cerevisiae</i>	12000 µg/ml			Mehta and von Borstel 1985
		pH 6.3	+	+	
		pH 7.0	-	+/-	
recombination	<i>Saccharomyces cerevisiae</i>	1000 µg/ml	-	-	Inge-Vechtomov <i>et al.</i> 1985
recombination	<i>Saccharomyces cerevisiae</i>	5000 µg/ml	-	-	Arni 1985
recombination	<i>Saccharomyces cerevisiae</i>	1000 µg/ml	-	-	Inge-Vechtomov <i>et al.</i> 1985
recombination	<i>Saccharomyces cerevisiae</i>	1000 µg/ml	-	-	Arni 1985
		5000 µg/ml	(+)	(+)	
recombination	<i>Saccharomyces cerevisiae</i>	2000 µg/ml	-	-	Brooks <i>et al.</i> 1985
recombination	<i>Saccharomyces cerevisiae</i>	5000 µg/ml	-	-	Parry and Eckardt 1985
recombination	<i>Saccharomyces cerevisiae</i>	12000 µg/ml			Mehta and von Borstel 1985
		pH 6.3	+	+	
		pH 7.0	-	-	
recombination	<i>Saccharomyces cerevisiae</i>	200000 µg/ml	-	-	Carls and Schiestl 1994
aneuploidy	<i>Saccharomyces cerevisiae</i> D6	5000 µg/ml	-	(+)	Parry and Eckardt 1985
mutation	<i>Saccharomyces pombe</i> P1	5900 µg/ml	?	-	Loprieno <i>et al.</i> 1985
mutation	<i>Aspergillus nidulans</i>	9900 µg/ml	-	n.d.	Carere <i>et al.</i> 1985
recombination	<i>Aspergillus nidulans</i>	9900 µg/ml	-	n.d.	Carere <i>et al.</i> 1985
nondisjunction	<i>Aspergillus nidulans</i>	9900 µg/ml	-	n.d.	Carere <i>et al.</i> 1985
Mammalian cells					
SCE	lymphocytes, human	1000 µg/ml	-	-	Obe <i>et al.</i> 1985
SCE	lymphocytes, human (metabolic activation by co-cultivation with rat liver cells)	39 µg/ml	-	(+)	Lindahl-Kiessling <i>et al.</i> 1989
SCE	CHO cells, Chinese hamster	390 µg/ml	-	n.d.	Abe and Sasaki 1977
SCE	CHO cells, Chinese hamster	3900 µg/ml	-	-	Douglas <i>et al.</i> 1985

Table 6. continued

Test system		Concentration ^a	Results ^b		References
			-S9	+S9	
SCE	CHO cells, Chinese hamster	5000 µg/ml	–	–	Gulati <i>et al.</i> 1985
SCE	RL4 cells, liver, rat	1000 µg/ml	–	n.d.	Priston and Dean 1985
DNA-SB (alkaline elution)	primary hepatocytes, rat	3900 µg/ml	–	n.d.	Bradley 1985
DNA-SB (comet assay)	primary hepatocytes, rat, Chinese hamster	9750 µg/ml	–	n.d.	Schmezer <i>et al.</i> 1988
DNA-SB (saccharose gradient)	CHO cells, Chinese hamster	3900 µg/ml	–	–	Douglas <i>et al.</i> 1985
DNA-SB (comet assay)	lymphocytes, human	195 µg/ml	+	n.d.	Anderson <i>et al.</i> 1999b
	leukocytes, human	3900 µg/ml	–	–	
DNA binding (³² P postlabelling)	primary hepatocytes, rat	390 µg/ml	–	n.d.	Gupta <i>et al.</i> 1985
UDS	primary hepatocytes, mouse	390 µg/ml	–	n.d.	Smith-Oliver and Butterworth 1987
UDS	primary hepatocytes, rat	986 µg/ml	–	n.d.	Astill <i>et al.</i> 1986
UDS	primary hepatocytes, rat	3900 µg/ml	–	n.d.	Butterworth <i>et al.</i> 1984
UDS	primary hepatocytes, rat	3900 µg/ml	–	n.d.	Kornbrust <i>et al.</i> 1984
UDS	primary hepatocytes, rat	3900 µg/ml	–	n.d.	Probst and Hill 1985
UDS	primary hepatocytes, rat	10000 µg/ml	–	n.d.	Williams <i>et al.</i> 1985
UDS	primary hepatocytes, human	3900 µg/ml	–	n.d.	Butterworth <i>et al.</i> 1984
TK ^{+/-}	L5178Y lymphoma cells, mouse	245 µl/ml	–	–	Astill <i>et al.</i> 1986
TK ^{+/-}	L5178Y lymphoma cells, mouse	400 µg/ml	–	(+)	Oberly <i>et al.</i> 1985
TK ^{+/-}	L5178Y lymphoma cells, mouse	2500 µg/ml	n.d.	–	Amacher and Turner 1985
TK ^{+/-}	L5178Y lymphoma cells, mouse	4900 µg/ml	–	–	Kirby <i>et al.</i> 1983
TK ^{+/-}	L5178Y lymphoma cells, mouse	4900 µg/ml	–	–	Myhr <i>et al.</i> 1985
TK ^{+/-}	L5178Y lymphoma cells, mouse	9800 µg/ml	–	–	Styles <i>et al.</i> 1985

Table 6. continued

Test system		Concentration ^a	Results ^b		References
			-S9	+S9	
TK ^{+/-}	TK6 cells, lymphoblasts, human	1000 µg/ml	-	-	Crespi <i>et al.</i> 1985
Na ⁺ /K ⁺ -ATPase	L5178Y lymphoma cells, mouse	200 µg/ml	-	-	Garner and Campbell 1985
Na ⁺ /K ⁺ -ATPase	L5178Y lymphoma cells, mouse	9800 µg/ml	-	-	Styles <i>et al.</i> 1985
Na ⁺ /K ⁺ -ATPase	BALB/c-3T3 cells, mouse	7910 µg/ml	n.d.	-	Matthews <i>et al.</i> 1985
HPRT	AHH-1 cells, lymphoblasts, human	1000 µg/ml	-	-	Crespi <i>et al.</i> 1985
CA	CHO cells, Chinese hamster	390 µg/ml	-	n.d.	Abe and Sasaki 1977
CA	CHO cells, Chinese hamster	780 µg/ml	-	n.d.	Phillips <i>et al.</i> 1982
CA	CHO cells, Chinese hamster	5000 µg/ml	-	-	Gulati <i>et al.</i> 1985
CA	CHL cells, Chinese hamster	4130 µg/ml	-	n.d.	Ishidate and Odashima 1977
CA	CHL cells, Chinese hamster	4130 µg/ml	-	-	Ishidate and Sofuni 1985
CA	CH1-L cells, liver, Chinese hamster	50 µg/ml	-	n.d.	Danford 1985
CA	SHE cells, embryo, Syrian hamster	≤ 1.8 µg/ml ≥ 0.9 µg/ml	- -	- +	Tsutsui <i>et al.</i> 1993
CA	RL4 cells, liver, rat	1000 µg/ml	-	n.d.	Priston and Dean 1985
CA	lymphocytes, human	60 µg/ml	-	n.d.	Stenchever <i>et al.</i> 1976
CA	lymphocytes, human	75 µg/ml	-	n.d.	Turner <i>et al.</i> 1974
CA	lymphocytes, human	160 µg/ml	-	n.d.	Tsuchiya and Hattori 1979
MN	CHO cells, Chinese hamster	3900 µg/ml	-	-	Douglas <i>et al.</i> 1985
MN	primary hepatocytes, rat	3900 µg/ml	-	n.d.	Müller-Tegethoff <i>et al.</i> 1995
MN	SHE cells, embryo, Syrian hamster	not stated (max. 10000 µg/ml)	+	n.d.	Fritzenschaf <i>et al.</i> 1993
aneuploidy (hyperploidy)	CHO cells, Chinese hamster	≤ 25 µg/ml 50 µg/ml	- +	n.d. n.d.	Danford 1985

Table 6. continued

Test system		Concentration ^a	Results ^b		References
			–S9	+S9	
aneuploidy	RL4 cells, liver, rat	1000 µg/ml	–	n.d.	Priston and Dean 1985
aneuploidy	lung cells, foetus, human	6 µg/ml	–	n.d.	Stenchever <i>et al.</i> 1976
disturbance of mitosis	CH1-L cells, liver, Chinese hamster	5 µg/ml	+	n.d.	Parry 1985
MEHP					
rec assay	<i>Bacillus subtilis</i>	≤ 300 µg/filter	–	n.d.	Tomita <i>et al.</i> 1982b
		≥ 400 µg/filter	+	n.d.	
BMT	<i>S. typhimurium</i> TA98, TA 100, TA1535, TA1537, TA1538	196 µg/plate	–	–	Kirby <i>et al.</i> 1983
BMT	<i>S. typhimurium</i> TA97, TA98, TA100, TA102	1000 µg/plate	–	–	Dirven <i>et al.</i> 1991
BMT	<i>S. typhimurium</i> TA100	1250 µg/plate	–	–	Tomita <i>et al.</i> 1982b
SCE	V79 cells, Chinese hamster	25 µg/ml	+	n.d.	Tomita <i>et al.</i> 1982b
DNA-SB (comet assay)	leukocytes, human	39 µg/ml	+	n.d.	Anderson <i>et al.</i> 1999b
UDS	primary hepatocytes, mouse	139 µg/ml	–	n.d.	Smith-Oliver and Butterworth 1987
UDS	primary hepatocytes, human	139 µg/ml	–	n.d.	Butterworth <i>et al.</i> 1984
TK ^{+/-}	L5178Y lymphoma cells, mouse	296 µg/ml	–	–	Kirby <i>et al.</i> 1983
CA	SHE cells, embryo, Syrian hamster	≤ 1.7 µg/ml	–	–	Tsutsui <i>et al.</i> 1993
		≥ 3.9 µg/ml	–	+	
Mono(2-ethyl-5-hydroxyhexyl)phthalate					
BMT	<i>S. typhimurium</i> TA97, TA98, TA100, TA102	1000 µg/plate	–	–	Dirven <i>et al.</i> 1991
Mono(2-ethyl-5-oxohexyl)phthalate					
BMT	<i>S. typhimurium</i> TA97, TA98, TA100, TA102	1000 µg/plate	–	–	Dirven <i>et al.</i> 1991
Mono(5-carboxyl-2-ethylpentyl)phthalate					
BMT	<i>S. typhimurium</i> TA97, TA98, TA100, TA102	1000 µg/plate	–	–	Dirven <i>et al.</i> 1991

^a lowest effective or highest ineffective concentration^b +: positive; (+): weakly positive; –: negative; n.d.: not determined

BMT: bacterial mutagenicity test; CA: test for structural chromosome aberrations; DNA-SB: test for DNA strand breaks; HPRT: HPRT gene mutation test; MN: micronucleus test; SCE: test for sister chromatid exchange; TK^{+/-}: TK^{+/-} mutation test; Na⁺/K⁺-ATPase: Na⁺/K⁺-ATPase gene mutation test; UDS: DNA repair synthesis test

DEHP was not found to cause an increase in the incidence of aneuploidy in either RL4 rat liver cells (Priston and Dean 1985) or human foetal lung cells (Stenchever *et al.* 1976). In CH1-L cells, after exposure to DEHP effects on the spindle apparatus were seen in the form of abnormal division stages, chromosomal clusters and chromosome lagging and bridges in the anaphase (Parry 1985).

MEHP, the main metabolite of DEHP, was not found to be mutagenic in the *Salmonella* mutagenicity test (Dirven *et al.* 1991, Kirby *et al.* 1983, Tomita *et al.* 1982b). In *Bacillus subtilis*, a concentration-dependent increase in recombinations was observed, which was interpreted as evidence of DNA-damaging properties of MEHP (Tomita *et al.* 1982b). An increase in the incidence of SCE was found in V79 cells (Tomita *et al.* 1982b) and human leukocytes (Anderson *et al.* 1999b) in the absence of S9 mix. In the UDS test with cultured hepatocytes from the mouse (Smith-Oliver and Butterworth 1987) and humans (Butterworth *et al.* 1984), DNA repair synthesis was not induced. A TK^{+/−} mutation test with L5178Y mouse lymphoma cells with MEHP yielded negative results (Kirby *et al.* 1983). In SHE cells, in the presence of S9 mix, but not in its absence, MEHP induced chromosomal aberrations (Tsutsui *et al.* 1993).

Mono(2-ethyl-5-hydroxyhexyl)phthalate, mono(2-ethyl-5-oxohexyl)phthalate and mono(5-carboxyl-2-ethylpentyl)phthalate were not found to be mutagenic in a *Salmonella* mutagenicity test (Dirven *et al.* 1991).

2-Ethylhexanol was not observed to have genotoxic effects in numerous studies (see the chapter “2-Ethylhexanol” in Volume 20 of the present series).

5.6.2 *In vivo* Studies

5.6.2.1 *Drosophila melanogaster*

Studies of the genotoxicity of DEHP in *Drosophila melanogaster* are listed in Table 7.

In two tests for somatic mutations and recombinations (eye mosaic test) DEHP produced weakly positive results (Fujikawa *et al.* 1985, Vogel 1985), in the less sensitive wing mosaic test negative results (Kawai 1998, Würgler *et al.* 1985). Negative results were also obtained in a test for recessive lethal mutations (Yoon *et al.* 1985, Zimmering *et al.* 1989).

Table 7. Studies of the genotoxicity of DEHP in *Drosophila melanogaster*

Test system	Concentration ^a	Results ^b	References
SMART (eye mosaic test)	780 µg/ml	(+)	Vogel 1985
SMART (eye mosaic test)	7800 µg/ml	(+)	Fujikawa <i>et al.</i> 1985
SMART (wing mosaic test)	78000 µg/ml	–	Würgler <i>et al.</i> 1985
SMART (wing mosaic test)	40 µl/bottle	–	Kawai 1998
SLRL	20 µg/ml	–	Yoon <i>et al.</i> 1985
SLRL	18600 µg/ml	–	Zimmering <i>et al.</i> 1989

^a lowest effective or highest ineffective concentration

^b +: positive; (+): weakly positive; –: negative

SLRL: test for recessive lethal mutations on the X chromosome

SMART: test for somatic mutations and recombinations

5.6.2.2 Mouse, rat, hamster

Studies of the genotoxicity of DEHP in the mouse, rat and hamster are listed in Table 8.

In the livers of rats exposed to DEHP neither an increase in the incidence of DNA strand breaks (Elliott and Elcombe 1987, Tamura *et al.* 1991) nor covalent DNA binding (Albro *et al.* 1982, von Däniken *et al.* 1984, Gupta *et al.* 1985, Lutz 1986) was detected. After rats were given DEHP with the diet for 2 weeks (Takagi *et al.* 1990b) and 1 year (Takagi *et al.* 1990a), an increased incidence of oxidative base damage, 8-oxoguanine, was found in the liver DNA. In another, very similar study, however, no increase in the formation of 8-oxoguanine in the DNA was detected (Cattley and Glover 1993). UDS tests with the livers of mice (Smith-Oliver and Butterworth 1987) and rats (Butterworth *et al.* 1984, Cattley *et al.* 1988, Kornbrust *et al.* 1984) did not yield evidence of induction of DNA repair synthesis by DEHP.

In a gene mutation test with transgenic mice (lacI system), DEHP did not induce detectable mutations (Gunz *et al.* 1993).

DEHP did not cause chromosomal aberrations in the bone marrow of rats (Putman *et al.* 1983), micronuclei in the erythrocytes of mice (Astill *et al.* 1986, Douglas *et al.* 1986) or aneuploidy in the liver of rats (Hasmall and Roberts 1997). In the embryos of female Syrian hamsters given single, high, oral doses of DEHP on day 11 of gestation, an increase in structural chromosomal aberrations was found (Tomita *et al.* 1982b).

In the coat colour spot test, DEHP, applied with the alkylating agent ethylnitrosourea (ENU), caused a marked increase in the recombinogenic effects of ENU and at the same time reduced the induction of gene mutations by ENU in two independent experiments with the mouse. The influence of DNA repair processes has been suggested as the reason for these effects of DEHP. DEHP alone was not used in these experiments. In view of the known, similar effects of tumour promoters such as 12-*O*-tetradecanoylphorbol-13-acetate and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, the co-recombinogenic effects of DEHP were regarded as evidence of its possible tumour-promoting effects (Fahrig and Steinkamp-Zucht 1996).

Table 8. *In vivo* studies of the genotoxicity of DEHP

Test system		Dose	Result ^a	References
DEHP				
DNA-SB, liver (alkaline elution)	rat, Wistar, groups of 4 ♂	28 days, 1000 mg/kg body weight and day, p.o.	–	Elliott and Elcombe 1987
	rat, F344, groups of 4–6 ♂, ♀	28 days, 2000 mg/kg body weight and day, p.o.	–	
DNA-SB, liver (alkaline elution)	rat, F344, 3–4 ♂	78 weeks, 20000 mg/kg diet (about 800 mg/kg body weight and day)	–	Tamura <i>et al.</i> 1991
oxidative DNA damage (8-Oxo-dG), liver, kidneys	rat, F344, groups of 3 ♂	1–2 weeks or 1 year, 12000 mg/kg diet (about 480 mg/kg body weight and day)	+ (liver) – (kidneys)	Takagi <i>et al.</i> 1990a, 1990b
oxidative DNA damage (8-Oxo-dG), liver	rat, F344, groups of 6 ♂	11 or 22 weeks, 12000 mg/kg diet	–	Cattley and Glover 1993
DNA binding, liver (bound radioactivity)	rat, F344, ♂	11 days, 100, 10000 mg/kg diet	–	Albro <i>et al.</i> 1982
DNA binding, liver (bound radioactivity)	rat, F344, 2 ♀	4 weeks, 10000 mg/kg diet	–	von Däniken <i>et al.</i> 1984
DNA binding, liver (bound radioactivity)	rat, F344, 2 ♀	4 weeks, 10000 mg/kg diet	–	Lutz 1986
DNA binding, liver (³² P postlabelling)	rat, F344, 3 ♂	3 days, 2000 mg/kg body weight and day, p.o.	–	Gupta <i>et al.</i> 1985
UDS, liver	mouse, B6C3F ₁ , groups of at least 3 ♂	28 days, 10, 100, 500 mg/ kg body weight and day, p.o.	–	Smith-Oliver and Butterworth 1987
		28 days, 6000 mg/kg diet	–	
UDS, liver	rat, F344, groups of 3 ♂, 3 ♀	1 × 500 mg/kg body weight, p.o. (♂, ♀)	–	Butterworth <i>et al.</i> 1984
		14 days, 150 mg/kg body weight and day, p.o. (♂)	–	
		30 days, 12000 mg/kg diet (♀)	–	
UDS, liver	rat, F344, groups of 4 ♂	28 days, 12000 mg/kg diet	–	Cattley <i>et al.</i> 1988
UDS, liver	rat, SD, ♂	1 × 5000 mg/kg body weight, p.o.	–	Kornbrust <i>et al.</i> 1984
gene mutation, liver	mouse, C57BL/6 (trans- genic, lacI), groups of 3 ♀	120 days, 3000, 6000 mg/kg diet	–	Gunz <i>et al.</i> 1993
CA, bone marrow	rat, F344, groups of 5 ♂	5 × 0.5, 1.7, 5.0 ml/kg body weight and day, p.o. (about 500, 1700, 5000 mg/kg body weight)	–	Putman <i>et al.</i> 1983

Table 8. continued

Test system		Dose	Result ^a	References
CA, embryo	Syrian hamster, ♀	1 × 3750 mg/kg body weight, GD 11, p.o.	(+)	Tomita <i>et al.</i> 1982b
		7500, 15000 mg/kg body weight, p.o.	+	
MN, no other details	mouse, no other details	1 × or 3 weeks 5000 mg/kg body weight and day, i.p.	–	Astill <i>et al.</i> 1986
MN, erythrocytes	mouse, B6C3F ₁ , groups of 3–5 ♂	5 × 600, 3000, 6000 mg/kg body weight and day, i.p.	–	Douglas <i>et al.</i> 1986
aneuploidy, liver	rat, F344, groups of 3 ♂	7 × 12000 mg/kg diet	–	Hasmall and Roberts 1997
coat colour spot test	mouse, C57BL × T-stock, 580 ♀	1 × 30 mg ENU/kg body weight + 5 ml (about 5000 mg) DEHP/kg body weight, GD 9, i.p.	weakening of mutagenic effect, amplification of re-combinogenic effect of ENU	Fahrig and Steinkamp-Zucht 1996
DLT	mouse, ICR Swiss, groups of 10 ♂	1 × 12800, 19200, 25600 mg/kg body weight, i.p., mating over 12 weeks with 2 untreated ♀ per week	+ ¹	Singh <i>et al.</i> 1974
DLT	mouse, no details, groups of at least 7 ♂	3 × 1000–100000 mg/kg body weight (10 dose groups), s.c., on days 1, 5, 10; mating on day 21	+ ¹	Autian 1982
DLT	mouse, ICR Swiss, groups of 8 ♂	3 × about 1000, 2000, 5000, 10000 mg/kg body weight, s.c., on days 1, 5, 10; mating on day 21	+ ¹	Agarwal <i>et al.</i> 1985
MEHP				
DNA-SB, liver (alkaline elution)	rat, Wistar, 4 ♂	28 days, 500 mg/kg body weight, p.o.	–	Elliott and Elcombe 1987
CA, embryo	Syrian hamster, ♀	1 × > 375 mg/kg body weight, GD 11, p.o.	+	Tomita <i>et al.</i> 1982b

^a +: positive; (+): weakly positive; –: negative¹ positive results caused by testicular toxicity (Adler and Ashby 1989)

CA: test for structural chromosomal aberrations; DLT: dominant lethal test; DNA-SB: test for DNA strand breaks; GD: day of gestation; MN: micronucleus test; UDS: test for DNA repair synthesis

A research group reported positive results in investigations into the possible mutagenic effects of DEHP on germ cells using the dominant lethal test (Autian 1982, Agarwal *et al.* 1985, Singh *et al.* 1974). In a later re-evaluation, these findings were attributed to the testicular toxicity of DEHP (Adler and Ashby 1989).

MEHP did not cause an increase in the incidence of DNA strand breaks in the livers of rats (Elliott and Elcombe 1987). However, like DEHP, it induced an increase in structural chromosomal aberrations in the embryos of female Syrian hamsters given single, high, oral doses of MEHP on day 11 of gestation (Tomita *et al.* 1982b).

2-Ethylhexanol did not cause the induction of micronuclei in micronucleus tests in the mouse (see the chapter “2-Ethylhexanol” in Volume 20 of the present series).

The available data for the genotoxicity of DEHP and its metabolites *in vitro* and *in vivo* do not indicate any clear genotoxic effects of the substances. In the case of the occasional positive results, for example the induction of SCE or DNA strand breaks *in vitro* or the increased formation of chromosomal aberrations in the embryos of Syrian hamsters after exposure to DEHP or MEHP *in utero*, the involvement of cytotoxic effects in the development of the genotoxic effects cannot be excluded.

5.7 Carcinogenicity

5.7.1 Short-term studies

5.7.1.1 Cell transformation tests

Numerous cell transformation tests with different test systems were carried out with DEHP (see Table 9).

In tests with mouse fibroblast cell lines (BALB/3T3, C3H/10T^{1/2}), DEHP increased the incidence of morphologically transformed clones in only one of the studies with C3H/10T^{1/2} cells (Lawrence and McGregor 1985). In the other studies with this cell line (Sanchez *et al.* 1987) and BALB/3T3 cells (Astill *et al.* 1986, Matthews *et al.* 1985) the substance had no transforming effects.

In cell transformation tests, in which primary embryo cells of the Syrian hamster (SHE cells) were used, DEHP induced transformations in all investigations carried out (Barrett and Lamb 1985, Dhalluin *et al.* 1998, Jones *et al.* 1988, Mikalsen and Sanner 1993, Mikalsen *et al.* 1990, Sanner and Rivedal 1985, Tsutsui *et al.* 1993). The transforming effects were observed even in the absence of an exogenous metabolic activation system. Positive results were obtained also in a transformation test with epidermal cells of the mouse (Diwan *et al.* 1985) and in two tests for the amplification of virus-induced cell transformation by DEHP (Hatch and Anderson 1985, Suk and Humphreys 1985).

Table 9. Cell transformation tests with DEHP

Test system	Concentration ^a	Results ^b		References
		– S9	+ S9	
DEHP <i>in vitro</i>				
BALB/3T3 fibroblasts, mouse	21 µg/ml	–	–	Astill <i>et al.</i> 1986
BALB/3T3 fibroblasts, mouse	21 µg/ml	–		Matthews <i>et al.</i>
	50000 µg/ml		–	1985
C3H/10T1/2 fibroblasts, mouse	39 µg/ml	–	n.d.	Sanchez <i>et al.</i> 1987
C3H/10T1/2 fibroblasts, mouse	20 µg/ml	–	+	Lawrence and
	40 µg/ml	–	+	McGregor 1985
	500 µg/ml			
	1000 µg/ml			
SHE cells, embryo, Syrian hamster	≤ 0.1 µg/ml	–	n.d.	Barrett and Lamb
	≥ 1.0 µg/ml	+		1985
SHE cells, embryo, Syrian hamster	1, 3.9 µg/ml	+	(+)	Tsutsui <i>et al.</i>
	≥ 10 µg/ml	(+)	+	1993
SHE cells, embryo, Syrian hamster	3.9 µg/ml	+	n.d.	Sanner and Rivedal 1985
SHE cells, embryo, Syrian hamster	3.9 µg/ml	+	n.d.	Mikalsen <i>et al.</i> 1990
SHE cells, embryo, Syrian hamster	39 µg/ml	+	n.d.	Dhalluin <i>et al.</i> 1998
SHE cells, embryo, Syrian hamster	30 µg/ml	+	n.d.	Mikalsen and Sanner 1993
SHE cells, embryo, Syrian hamster	63 µg/ml	+	n.d.	Jones <i>et al.</i> 1988
SHE cells, embryo, Syrian hamster (test for amplification of the cell transformation caused by the adenovirus SA7)	≤ 234 µg/ml	–	n.d.	Hatch and Anderson 1985
	≥ 500 µg/ml	+		
embryo cells, Fischer rat (test for amplification of the cell transformation caused by the Rauscher leukaemia virus)	≤ 1000 µg/ml	–	n.d.	Suk and Humphreys 1985
	≥ 2000 µg/ml	+		
JB6 cells, epidermis cells, mouse	500 µg/ml	+	n.d.	Diwan <i>et al.</i> 1985
DEHP <i>in vivo</i>				
SHE cells, embryo, Syrian hamster, treated <i>in utero</i>	1 × 7500 mg/kg body weight, GD 11, p.o.	+	n.d.	Tomita <i>et al.</i> 1982b
MEHP <i>in vitro</i>				
C3H/10T1/2 fibroblasts, mouse	1500 µM	–	n.d.	Sanchez <i>et al.</i> 1987

Table 9. continued

Test system	Concentration ^a	Results ^b		References
		– S9	+ S9	
SHE cells, embryo, Syrian hamster	10 µM 200 µM	(+)	+	Tsutsui <i>et al.</i> 1993
Syrian hamster, primary embryo cells, clonal assay	10 µM	(+)	n.d.	Mikalsen <i>et al.</i> 1990
MEHP <i>in vivo</i>				
SHE cells, embryo, Syrian hamster, treated <i>in utero</i>	1 × 375 mg/kg body weight, GD 11, p.o.	+	n.d.	Tomita <i>et al.</i> 1982b

^a lowest effective or highest ineffective concentration

^b +: positive; (+): weakly positive; –: negative; n.d.: not determined
GD: day of gestation

Also MEHP induced cell transformation in SHE cells (Mikalsen *et al.* 1990, Tsutsui *et al.* 1993), but not in C3H/10T½ mouse fibroblasts (Sanchez *et al.* 1987).

In an *in vivo* test, an increase in the incidence of transformations was observed in SHE cells from the embryos of female Syrian hamsters given single, high, oral doses of DEHP or MEHP on day 11 of gestation (Tomita *et al.* 1982b).

The mechanism of the transforming effects of DEHP and MEHP is unclear. In view of the mainly negative results for the genotoxicity of DEHP and MEHP, a non-genotoxic mechanism of action is probably responsible for the positive results in cell transformation tests.

5.7.1.2 Tests for the blocking of intercellular communication channels (gap junctions)

The tests carried out for the inhibition of intercellular communication via the appropriate channels are listed in Table 10.

In SHE cells (Cruciani *et al.* 1997, Mikalsen and Sanner 1993), V79 cells (Cruciani *et al.* 1997, Elmore *et al.* 1985, Malcolm and Mills 1989, Malcolm *et al.* 1983, Vang *et al.* 1993) and TM4 Sertoli cells of the mouse (Kang *et al.* 2002), DEHP caused the inhibition of intercellular communication. Only in an investigation with V79 cells were no inhibitory effects found (Kornbrust *et al.* 1984). The inhibitory effects of DEHP, like the transforming effects (see above), occurred even in the absence of an exogenous metabolic activation system.

Inhibition of intercellular communication in tissue sections of the liver was found in F344 rats and B6C3F₁ mice after treatment with DEHP for 2 weeks (Isenberg *et al.* 2000), but not in Syrian hamsters (Isenberg *et al.* 2000) or cynomolgus monkeys (Pugh *et al.* 2000). Intercellular communication in the liver was reversible in rats 2 weeks after the end of exposure and similar to that in untreated animals (Isenberg *et al.* 2001). The inhibition of intercellular communication by DEHP observed in the *in vitro* experiments indicates DEHP may have tumour-promoting properties.

Table 10. Tests for the blocking of intercellular communication channels (gap junctions)

Test system	Concentration ^a	Results ^b		References
		– S9	+ S9	
<i>In vitro</i>				
V79 cells, Chinese hamster	0.1 µg/ml	–	n.d.	Kornbrust <i>et al.</i> 1984
V79 cells, Chinese hamster	5 µg/ml	+	n.d.	Elmore <i>et al.</i> 1985
V79 cells, Chinese hamster	10 µg/ml	+	n.d.	Malcolm <i>et al.</i> 1983
V79 cells, Chinese hamster	10 µg/ml	+	n.d.	Malcolm and Mills 1989
V79 cells, Chinese hamster	78 µg/ml	+	n.d.	Vang <i>et al.</i> 1993
V79 cells, Chinese hamster	30 µg/ml	+	n.d.	Cruciani <i>et al.</i> 1997
SHE cells, embryo, Syrian hamster	30 µg/ml	+	n.d.	Mikalsen and Sanner 1993
TM4 Sertoli cells, mouse	39 µg/ml	+	n.d.	Kang <i>et al.</i> 2002
	195 µg/ml	(+)		
<i>In vivo</i>				
liver, rat, F344, groups of 5 ♂	2 weeks, 1000, 20000 mg/kg diet	+		Isenberg <i>et al.</i> 2000
	followed by 1 or 2 weeks control diet	–		Isenberg <i>et al.</i> 2001
liver, mouse, B6C3F ₁ , groups of 5 ♂	2, 4 weeks, 500, 6000 mg/kg diet	+		Isenberg <i>et al.</i> 2000
liver, Syrian hamster, groups of 5 ♂	2 weeks, 1000, 6000 mg/kg diet	–		Isenberg <i>et al.</i> 2000
liver, monkey, cynomolgus, groups of 4 ♂	2 weeks, 500 mg/kg body weight and day, p.o.	–		Pugh <i>et al.</i> 2000

^a lowest effective or highest ineffective concentration^b +: positive; (+): weakly positive; –: negative; n.d.: not determined

5.7.1.3 Initiation–promotion studies

In numerous initiation–promotion studies the initiating or promoting effects of DEHP on the liver (Table 11) and on the kidneys, bladder and skin (Table 12) were investigated.

Liver: DEHP was not found to have initiating effects on the liver in either F344 rats (Garvey *et al.* 1987) or B6C3F₁ mice (Ward *et al.* 1986). Marked promoting effects of DEHP on the liver after initiation with diethylnitrosamine were observed in male B6C3F₁ mice (Hagiwara *et al.* 1986, Ward *et al.* 1983, 1984, 1986). In Sprague-Dawley rats DEHP was found to be only a weak promoter in the liver (Gerbracht *et al.* 1990, Oesterle and Deml 1988) and in F344 rats not to be a promoter (Ito *et al.* 1988, Popp *et al.* 1985, Ward *et al.* 1990).

Table 11. Initiation–promotion studies with DEHP in the liver of rats and mice

Test system	Initiation	Promotion	DEHP effect	References
rat, F344, groups of 10 ♂	PH , 1 × 10000 mg DEHP /kg body weight, p.o.	2 weeks, 0.02 % AAF with the diet + 1 × 1.5 ml CCl₄ /kg body weight, p.o.	– (not an initiator)	Garvey <i>et al.</i> 1987
rat, F344, groups of 10 ♀	12 weeks, 12000 mg DEHP /kg diet	39 weeks, 0.05 % PB with the diet	– (not an initiator)	Garvey <i>et al.</i> 1987
rat, F344, 20 ♂	1 × 200 mg DEN /kg body weight, i.p., PH	6 weeks, 3000 mg DEHP /kg diet	– (not a promoter)	Ito <i>et al.</i> 1988
rat, F344, groups of 10 ♀	1 × 150 mg DEN /kg body weight, i.p.	3 or 6 months, 12000 mg DEHP /kg diet	– (not a promoter)	Popp <i>et al.</i> 1985
rat, F344, groups of 10 ♀	1 × 282 mg DEN /kg body weight, i.p.	14 weeks, 12000 mg DEHP /kg diet	– (not a promoter)	Ward <i>et al.</i> 1990
rat, F344, groups of 12 ♀	1 × 200 mg DEN /kg body weight, i.p., PH	46 weeks, 30, 300, 3000, 12000 mg DEHP /kg diet	+ (promoter) foci after 300 mg/kg or more; tumours at 12000 mg/kg	Sano <i>et al.</i> 1999
rat, SD, groups of 5 ♀	1 × 8 mg DEN /kg body weight, p.o.	11 weeks (3 days/week), 10, 100, 200, 500 mg DEHP /kg body weight and day, p.o.	(+) (promoter)	Oesterle and Deml 1988
rat, SD, groups of 9–10 ♀	1 × 30 mg DEN /kg body weight, p.o.	11 weeks (3 days/week), 50, 200, 500, 1000, 2000 mg DEHP /kg body weight and day, p.o.	(+) (promoter)	Gerbracht <i>et al.</i> 1990
rat, SD, groups of 7–10 ♂	PH , 1 × 30 mg DEN /kg body weight, p.o.	7 weeks (3 days/week), 50, 200, 500, 1000, 2000 mg DEHP /kg body weight and day, p.o.	(+) (promoter)	Gerbracht <i>et al.</i> 1990
mouse, B6C3F ₁ , groups of 10 ♂	1 × 25, 50 mg DEHP /kg body weight, p.o.	6 months, 500 mg PB /l drinking water	– (not an initiator)	Ward <i>et al.</i> 1986
mouse, B6C3F ₁ , groups of 10 ♂	1 × 80 mg DEN /kg body weight, i.p.	6 months, 3000, 6000 mg DEHP /kg diet	+ (promoter)	Ward <i>et al.</i> 1983
mouse, B6C3F ₁ , groups of 10 ♂	1 × 80 mg DEN /kg body weight, i.p.	24 weeks, 3000 mg DEHP /kg diet	+ (promoter)	Ward <i>et al.</i> 1984

Table 11. continued

Test system	Initiation	Promotion	DEHP effect	References
mouse, B6C3F ₁ , groups of 10 ♂	1 × 80 mg DEN /kg body weight, i.p.	18 months, 3000, 6000, 12000 mg DEHP /kg diet	+ (promoter)	Ward <i>et al.</i> 1986
mouse, B6C3F ₁ , groups of 30 ♂	1 × 80 mg DEN /kg body weight, i.p.	29 weeks, 6000 mg DEHP /kg diet	+ (promoter)	Hagiwara <i>et al.</i> 1986

AAF: 2-acetylaminofluorene; DEN: diethylnitrosamine; PH: partial hepatectomy; PB: phenobarbital; SD: Sprague-Dawley

Table 12. Initiation–promotion studies with DEHP in the kidneys and bladder of rats and the skin of mice

Test system	Initiation	Promotion	DEHP effect	References
Kidneys				
rat, F344, groups of 20 ♂	2 weeks, 0.05 % N-ethyl-N-hydroxyethyl-nitrosamine , p.o.	24 weeks, 3500, 12000 mg DEHP /kg diet	+ (promoter)	Kurokawa <i>et al.</i> 1988
Bladder				
rat, F344, groups of 30 ♂	4 weeks, 0.05 % N-butyl-N-(4-hydroxybutyl)-nitrosamine in drinking water	weeks 5–8 + 12–20, 3000, 6000 or 12000 mg DEHP /kg diet; weeks 9–11, 3000 mg uracil /kg diet	– (not a promoter)	Hagiwara <i>et al.</i> 1990
Skin				
mouse, SENCAR, groups of 25 ♀	1 × 20 µg DMBA , epicutaneous	4 × 2 µg TPA , epicutaneous; 26 weeks, 100 µg DEHP (2×/week), epicutaneous	+ (in combination with TPA promoting effect)	Diwan <i>et al.</i> 1985, Ward <i>et al.</i> 1986
mouse, SENCAR, groups of 25 ♀	1 × 20 µg DMBA , epicutaneous	28 weeks, 100 µg DEHP (2×/week), epicutaneous	– (not a promoter)	Diwan <i>et al.</i> 1985, Ward <i>et al.</i> 1986
mouse, CD-1, groups of 20 (no other details)	1 × 50 µg DMBA , epicutaneous	40 weeks 98 µg DEHP (2×/week), epicutaneous	– (not a promoter)	Ward <i>et al.</i> 1986

DMBA: dimethylbenz[a]anthracene; TPA: 12-*O*-tetradecanoylphorbol-13-acetate

Kidneys: DEHP was found to be a tumour promoter in the kidneys of male F344 rats (Kurokawa *et al.* 1988).

Bladder: In rats, DEHP was not found to have tumour-promoting effects on the bladder (Hagiwara *et al.* 1990).

Skin: In skin-painting studies, DEHP was found to have promoting effects in SENCAR mice in a two-stage promotion experiment together with TPA (Diwan *et al.* 1985, Ward *et al.* 1986), but not in CD-1 mice (Ward *et al.* 1986).

5.7.1.4 Short-term carcinogenicity studies

Short-term carcinogenicity studies are shown in Table 13.

In the model of the neonatal mouse, a test for genotoxic carcinogens, DEHP did not cause any tumours (McClain *et al.* 2001).

In another short-term carcinogenicity test for genotoxic carcinogens with C57BL/6 wild type or transgenic mice (C57BL/6XPA^{-/-}, C57BL/6XPA^{-/-}p53^{+/-}), in both wild type and transgenic mice DEHP caused changes in the weights of the testes, liver and kidneys, and non-neoplastic changes in the testes, but no tumours (Mortensen *et al.* 2002).

In a test with transgenic mice with the human prototype *c-Ha-ras* gene (rasH2), changes were observed in the liver (foci, hypertrophy, deposition of pigments), kidneys (hydronephrosis, tubular regeneration) and nasal cavity (eosinophilic bodies) in wild-type mice and transgenic mice of both sexes after administration of DEHP with the diet for 26 weeks. Only in male transgenic mice was there a dose-dependent increase in hepatocellular adenomas. Molecular analysis of the adenomas did not reveal point mutations. The authors do not attribute the adenomas to the transgenic properties (Toyosawa *et al.* 2001).

Table 13. Short-term carcinogenicity studies in rats and mice with DEHP

Author:	McClain <i>et al.</i> 2001
Test system:	model of the neonatal mouse (NCTP protocol) (test for genotoxic carcinogens)
Substance:	DEHP; positive controls: 6-nitrochrysene
Species:	mouse, C57BL/6N
Administration route:	intraperitoneal, 2×
Amount:	5000, 10000 nmol (2, 4 mg)
Duration:	1 year
Toxicity:	not stated
Tumours:	no tumours

Table 13. continued

Author:	Mortensen <i>et al.</i> 2002				
Test system:	transgenic mice (C57BL/6XPAXpa ^{-/-} , C57BL/6Xpa ^{-/-} /p53 ^{+/-}) (test for genotoxic carcinogens)				
Substance:	DEHP; positive controls: <i>p</i> -cresidine				
Species:	mouse, C57BL/6N (wild-type, WT, 2 groups of 15 ♂, 15 ♀), C57BL/6XPAXpa ^{-/-} (XPA, 4 groups of 15 ♂, 15 ♀), C57BL/6Xpa ^{-/-} /p53 ^{+/-} (XPA/p53, 2 groups of 15 ♂, 15 ♀)				
Administration route:	with the diet				
Concentration:	0, 1500 (only XPA), 3000 (only XPA), 6000 mg/kg diet (about 200, 400, 880 mg/kg body weight and day)				
Duration:	39 weeks				
Toxicity:	≥ 1500 mg/kg diet (XPA): body weight gains decreased, relative water consumption increased, absolute and relative testis and liver weights decreased, absolute kidney weights decreased; 6000 mg/kg diet: survival decreased (♂: XPA, XPA/p53), absolute and relative testis weights decreased, absolute kidney weights decreased (WT, XPA/p53)				

	Concentration of DEHP in the diet (mg/kg)				

		0	1500	3000	6000

Male mouse					
testes					
interstitial hyperplasia	XPA	0/15	1/15	5/15	15/15*
	WT	0/15	—	—	10/14*
	XPA/p53	0/15	—	—	15/15*
tubular atrophy	XPA	9/15	14/15	15/15*	15/15*
	WT	8/15	—	—	13/14*
	XPA/p53	4/15	—	—	15/15*
Tumours:	none				

* statistically significantly different from the control group

Table 13. continued

Preneoplasms, tumours:

		Concentration of DEHP in the diet (mg/kg)			
		0	1500	3000	6000
Male mouse					
liver					
foci	rasH2	0/15	1/15 (7 %)	0/15	0/15
	non-Tg	0/15	—	—	0/15
hypertrophy	rasH2	0/15	9/15 (60 %)*	15/15 (100 %)*	15/15 (100 %)*
	non-Tg	0/15	—	—	15/15 (100 %)*
deposition of pigments	rasH2	0/15	0/15	2/15 (13 %)	8/15 (53 %)*
	non-Tg	0/15	—	—	11/15 (73 %)*
adenomas	rasH2	0/15	1/15 (7 %)	2/15 (13 %)	4/15 (27 %)*
	non-Tg	0/15	—	—	0/15
kidneys					
hydronephrosis	rasH2	0/15	0/15	0/15	1/15 (7 %)
	non-Tg	0/15	—	—	0/15
tubular regeneration	rasH2	8/15 (53 %)	3/15 (20 %)	5/15 (33 %)	12/15 (80 %)
	non-Tg	0/15	—	—	10/15 (67 %)*
testes					
focal atrophy	rasH2	4/15 (27 %)	5/15 (33 %)	11/15 (73 %)*	15/15 (100 %)*
	non-Tg	1/15 (7 %)	—	—	15/15 (100 %)*
nasal cavity					
eosinophilic bodies	rasH2	4/15 (27 %)	—	—	11/15 (73 %)*
	non-Tg	1/15 (7 %)	—	—	9/15 (60 %)*
Female mouse					
liver					
foci	rasH2	0/15	0/15	0/15	1/15 (7%)
	non-Tg	0/15	—	—	0/15
hypertrophy	rasH2	0/15	15/15 (100 %)*	15/15 (100 %)*	15/15 (100 %)*
	non-Tg	0/15	—	—	15/15 (100 %)*
deposition of pigments	rasH2	0/15	0/15	4/15 (27 %)*	15/15 (100 %)*
	non-Tg	0/15	—	—	15/15 (100 %)*
kidneys					
hydronephrosis	rasH2	0/15	0/15	0/15	2/15 (13 %)
	non-Tg	0/15	—	—	6/15 (40 %)*
tubular regeneration	rasH2	2/15 (13 %)	1/15 (7 %)	3/15 (20 %)	13/15 (87 %)*
	non-Tg	0/15	—	—	11/15 (73 %)*
nasal cavity					
eosinophilic bodies	rasH2	10/15 (67 %)	—	—	15/15 (100 %)*
	non-Tg	0/15	—	—	12/15 (80 %)*

* statistically significantly different from control group

5.7.2 Long-term studies

The most important studies of the carcinogenicity of DEHP are summarized in Table 14.

In an NTP carcinogenicity study, **F344 rats** were given doses of DEHP of 0, 6000 or 12000 mg/kg diet for 103 weeks. Survival, which at the end of the study was over 60 %, was not found to be affected by the substance. Body weight gains were reduced in both sexes. At the end of the study neoplastic changes were found in the liver in both sexes. In the male animals of the highest dose group (corresponding to 674 mg/kg body weight and day) the incidence of adenomas and carcinomas was significantly increased. For adenomas and carcinomas alone, the increase in the incidence was not significant. In the female animals, the incidence of adenomas and carcinomas was significantly increased even at the low dose (corresponding to 394 mg/kg body weight and day), as were the incidences for adenomas and carcinomas alone in the high dose group. Significantly reduced tumour incidences were observed in male animals of the high dose group for C cell adenomas and carcinomas of the thyroid gland and for Leydig cell tumours in the testes. The incidence of mononuclear cell leukaemia was slightly increased in both sexes, but not significantly so (NTP 1982).

In another carcinogenicity study with **F344 rats**, groups of 50 to 80 animals of each sex were exposed to doses of DEHP of 100, 500, 2500 or 12500 mg/kg diet for 104 weeks. In addition, a recovery group was exposed to doses of 12500 mg/kg diet for 78 weeks and then put on the control diet for another 26 weeks. In addition to the classical histological evaluation, at certain intervals DEHP-induced cell and peroxisome proliferation was determined. Survival was over 65 % in both sexes in all groups. A transient increase in cell proliferation in the liver was determined in the initial phase of the study, but was no longer detectable at weeks 79 and 105. After doses of 2500 mg/kg diet or more, increased liver weights and peroxisome proliferation were still determined at the end of the study. These effects were not observed in the recovery group. In male rats there was a significant increase in *spongiosis hepatis* (now known by its synonym pericytoma) after doses of 2500 mg/kg diet or more, and in addition adenomas and carcinomas of the liver and mononuclear cell leukaemia were observed. This type of leukaemia is a frequent spontaneous tumour in this strain of rat, but the incidence in this dose group (49 %) was greatly above that in the historical controls (30.5 %). At doses of 12500 mg/kg diet there was also an increase in pancreas adenomas. The incidence of Leydig cell tumours was decreased. Adenomas and carcinomas of the liver were significantly increased in the female animals of the lowest dose group (100 mg/kg diet) and highest dose group (12500 mg/kg diet); the finding in the lowest dose group is regarded as not substance-related as this effect was not seen in the two middle dose groups. Because of the lack of a dose–response relationship, a significantly increased incidence of mononuclear cell leukaemia only in the female animals of the lowest dose group is not regarded as relevant to the evaluation. The authors find it conspicuous that in the recovery group the incidences for adenomas and carcinomas of the liver are lower than in the highest dose group. Of importance for the mechanistic interpretation of the data is the coincidence between peroxisome proliferation and tumour development (David *et al.* 1999, 2000a).

Table 14. Long-term carcinogenicity studies in rats and mice with DEHP

Author:	Kluwe 1986, Kluwe <i>et al.</i> 1982, NTP 1982		
Substance:	DEHP (purity > 99.5 %)		
Species:	rat, F344, groups of 50 ♂, 50 ♀		
Administration route:	with the diet		
Concentration:	0, 6000, 12000 mg/kg diet (♂ about 0, 322, 674 mg/kg body weight and day; ♀ about 0, 394, 774 mg/kg body weight and day)		
Duration:	103 weeks		
Toxicity:	≥ 6000 mg/kg diet (322 mg/kg body weight and day) ♂: body weight gains slightly decreased; ♂, ♀: changes in clear cells of the liver; 12000 mg/kg diet (674 mg/kg body weight and day) ♂: hypertrophy of the pituitary gland, degeneration of the seminal vesicles in the testes; ♀: body weight gains slightly decreased		
Tumours:			

	Concentration of DEHP in the diet (mg/kg)		

	0 [historical control data]	6000	12000

Male rat			
liver			
neoplastic nodules (adenomas)	2/50 (4 %)	5/49 (10 %)	7/49 (14 %)
carcinomas	1/50 (2 %) [0.8 ± 1.1 %]	1/49 (2 %)	5/49 (10 %)
adenomas and carcinomas	3/50 (6 %) [4.2 ± 3.9 %]	6/49 (12 %)	12/49 (24 %)*
pituitary gland			
carcinomas and adenomas	8/46 (17 %)	6/43 (14 %)	1/49 (2 %)*
thyroid gland			
C cell carcinomas and adenomas	5/48 (10 %)	2/47 (4 %)	0/46 (0 %)*
testes			
Leydig cell tumours	47/49 (96 %)	42/44 (95 %)	11/48 (23 %)*
mononuclear cell leukaemia	13/50 (26 %)	20/50 (40 %)	17/50 (34 %)
Female rat			
liver			
neoplastic nodules (adenomas)	0/50 (0 %)	4/49 (8 %)	5/50 (10 %)*
carcinomas	0/50 (0 %) [0.2 ± 0.7 %]	2/49 (4 %)	8/50 (16 %)*
adenomas and carcinomas	0/50 (0 %) [3.1 ± 3.2 %]	6/49 (12 %)*	13/50 (26 %)*

Table 14. continued

Concentration of DEHP in the diet (mg/kg)						
	0 [historical control data]	6000	12000			
mononuclear cell leukaemia	10/50 (20 %)	14/50 (28 %)	17/50 (34 %)			
* statistically significantly different from control group						
Author:	David <i>et al.</i> 1999, 2000a					
Substance:	DEHP (purity 99.7 %)					
Species:	rat, F344, groups of 50 to 80 ♂, ♀					
Administration route:	with the diet					
Concentration:	0, 100, 500, 2500, 12500 mg/kg diet (♂ about 0, 6, 29, 147, 789 mg/kg body weight and day; ♀ about 0, 7, 36, 182, 939 mg/kg body weight and day)					
Duration:	104 weeks; recovery group: 78 weeks 12500 mg/kg diet, 26 weeks control diet					
Toxicity:	<p>≥ 100 mg/kg diet (6 mg/kg body weight and day) ♂: kidneys (age-related mineralization of the renal papillae increased)</p> <p>≥ 500 mg/kg diet (29 mg/kg body weight and day) ♂: testes (age-related bilateral aspermatogenesis increased)</p> <p>≥ 2500 mg/kg diet (147 mg/kg body weight and day) ♂: liver (absolute and relative liver weights increased, palmitoyl-CoA oxidase activity increased), lungs (relative lung weights increased), kidneys (absolute and relative kidney weights increased, mineralization and chronic nephropathy increased), mononuclear leukaemia increased; ♀: liver (absolute and relative liver weights increased, palmitoyl-CoA oxidase activity increased)</p> <p>12500 mg/kg diet (789 mg/kg body weight and day) ♂: relative lung weights increased, relative kidney weights increased, absolute and relative liver weights increased, palmitoyl-CoA oxidase increased; ♀: absolute and relative liver weights increased, palmitoyl-CoA oxidase increased ♂, ♀</p>					
Tumours:						
Concentration of DEPH in the diet (mg/kg)						
	0 [historical control data]	100	500	2500	12500	12500 recovery group
Male rats						
liver						
<i>spongiosis hepatis</i>	3/80 (4 %)	3/50 (6 %)	3/55 (6 %)	11/65 (17 %)*	11/80 (14 %)*	not stated
adenomas	4/80 (5 %)	5/50 (10 %)	3/55 (5 %)	8/65 (12 %)	21/80 (30 %) [#]	12/55 (22 %)

Table 14. continued

	Concentration of DEHP in the diet (mg/kg)					
	0 [historical control data]	100	500	2500	12500	12500 recovery group
carcinomas	1/80 (1 %)	0/50 (0 %)	1/55 (2 %)	3/65 (5 %)	24/80 (34 %) #	7/55 (13 %) #
adenomas and carcinomas	5/80 (7 %) [11/232, 4.7 %]	5/50 (10 %)	4/55 (7 %)	11/65 (17 %) *	34/80 (43 %) *	18/55 (33 %) *
pancreas, adenomas	0/60 (0 %)	0/17 (0 %)	0/14 (0 %)	0/18 (0 %)	5/59 (8 %) *	not stated
testes, Leydig cell tumours	59/64 (92 %)	45/50 (90 %)	50/55 (91 %)	60/65 (92 %)	20/64 (31 %) *	not stated
mononuclear cell leukaemia	15/65 (23 %) [128/420, 30.5 %]	13/50 (26 %)	16/55 (27 %)	32/65 (49 %) *	27/65 (42 %) *	29/55 (53 %) *
Female rats						
liver						
<i>spongiosis hepatis</i>	0/80 (0 %)	0/50 (0 %)	0/55 (0 %)	1/65 (2 %)	1/80 (1 %)	not stated
adenomas	0/80 (0 %)	3/50 (6 %)	1/55 (2 %)	2/65 (3 %)	8/80 (11 %)	6/55 (11 %)
carcinomas	0/80 (0 %)	1/50 (2 %)	0/55 (0 %)	1/65 (2 %)	14/80 (20 %) #	4/55 (7 %)
adenomas and carcinomas	0/80 (0 %) [4/329, 1.3 %]	4/50 (8 %) *	1/55 (2 %)	3/65 (5 %)	22/80 (31 %) *	10/55 (18 %) *
pancreas, adenomas	0/60 (0 %)	0/7 (0 %)	0/10 (0 %)	0/14 (0 %)	2/60 (3 %)	not stated
mononuclear cell leukaemia	14/65 (22 %) [82/424, 19.3 %]	17/50 (34 %) +	11/55 (20 %)	16/65 (25 %)	17/65 (26 %)	18/55 (33 %)

* statistically significantly different from control group; # statistically significantly different from control group, MAK calculation; + statistically significantly different from the historical controls

Author:	Berger 1996
Substance:	DEHP ("pure")
Species:	rat, Sprague-Dawley, 390 ♂ (controls), 60 ♂ (low dose), 100 ♂ (middle dose), 180 ♂ (high dose)
Administration route:	with the diet
Dose:	0, 600, 1897, 6000 mg/kg diet (6 days/week) (about 0, 30, 95, 300 mg/kg body weight and day)
Duration:	lifetime (max. 159 weeks)
Toxicity:	6000 mg/kg diet: spermiogenesis decreased
Tumours:	general and additional histology

Table 14. continued

Concentration of DEPH in the diet (mg/kg)				
	0	600	1897	6000
Male rats				
liver				
<i>spongiosis hepatis</i>	40/167 (24.0 %)	18/84 (21.4 %)	10/53 (18.9 %)	9/31 (29.0 %)
preneoplastic foci	63/167 (37.7 %)	32/84 (38.1 %)	20/53 (37.7 %)	7/31 (22.6 %)
adenomas	22/167 (13.2 %)	6/84 (7.1 %)	5/53 (9.4 %)	6/31 (19.4 %)
carcinomas	11/167 (6.6 %)	7/84 (8.3 %)	1/53 (1.9 %)	6/31 (19.4 %)*
adenomas and carcinomas	33/167 (19.8 %)	13/84 (15.5 %)	6/53 (11.3 %)*	12/31 (38.7 %)*
testes, Leydigomas	64/390 (16.4 %)	34/180 (18.9 %)	21/100 (21.0 %)	17/60 (28.3 %)*
* statistically significantly different from control group				
Author:	Kluwe 1986, Kluwe <i>et al.</i> 1982, NTP 1982			
Substance:	DEHP (purity > 99.5 %)			
Species:	mouse, B6C3F ₁ , groups of 50 ♂, 50 ♀			
Administration route:	with the diet			
Concentration:	0, 3000, 6000 mg/kg diet (♂ about 0, 672, 1325 mg/kg body weight and day; ♀ about 0, 799, 1821 mg/kg body weight and day)			
Duration:	103 weeks			
Toxicity:	≥ 3000 mg/kg diet (799 mg/kg body weight) ♀: body weight gains decreased; 6000 mg/kg diet (1325 mg/kg body weight) ♂: chronic nephropathy, degeneration of the seminal vesicles in the testes			
Tumours:				
Concentration of DEPH in the diet (mg/kg)				
	0 [historical control data]	3000	6000	
Male mice				
liver				
adenomas	6/50 (12 %)	11/48 (23 %)	10/50 (20 %)	
carcinomas	9/50 (18 %) [21.3 ± 6.9 %]	14/48 (29 %)	19/50 (38 %)*	
adenomas and carcinomas	14/50 (28 %) [31.1 ± 7.5 %]	25/48 (52 %)*	29/50 (58 %)*	
Female mice				
liver				
adenomas	1/50 (2 %)	5/50 (10 %)	1/50 (2 %)	
carcinomas	0/50 (0 %) [4.1 ± 3.0 %]	7/50 (14 %)*	17/50 (34 %)*	

Table 14. continued

	Concentration of DEHP in the diet (mg/kg)		
	0 [historical control data]	3000	6000
adenomas and carcinomas	1/50 (2 %) [7.9 ± 4.6 %]	12/50 (24 %)*	18/50 (36 %)*

* statistically significantly different from control group

Author:	David <i>et al.</i> 1999
Substance:	DEHP (purity 99.7 %)
Species:	mouse, B6C3F ₁ , groups of 65 ♂, 65 ♀
Administration route:	with the diet
Concentration:	0, 100, 500, 1500, 6000 mg/kg diet (♂ about 0, 19, 99, 292, 1266 mg/kg body weight and day; ♀ about 0, 24, 117, 354, 1458 mg/kg body weight and day)
Duration:	104 weeks; recovery group: 78 weeks 6000 mg/kg diet, 26 weeks control diet
Toxicity:	<p>≥ 500 mg/kg diet (99 mg/kg body weight) ♂: liver (palmitoyl-CoA oxidase activity increased, absolute liver weights increased), kidneys (relative kidney weights decreased), testes (relative testis weights decreased); ♀: liver (palmitoyl-CoA oxidase activity increased)</p> <p>≥ 1500 mg/kg diet (292 mg/kg body weight) ♂: liver (relative liver weights increased), kidneys (absolute kidney weights decreased, chronic progressive nephropathy increased), testes/epididymis (absolute testis weights decreased, bilateral hypospermia increased, immature or abnormal sperms in the epididymis); ♀: liver (relative liver weights increased)</p> <p>≥ 6000 mg/kg diet (1266 mg/kg body weight) ♂: body weight gains decreased, survival decreased, liver (hepatocellular pigmentation, cytoplasmatic eosinophilia, chronic inflammation), kidneys (absolute kidney weights decreased, chronic progressive nephropathy increased), lungs (relative lung weights increased), brain (relative brain weights increased); ♀: liver (absolute liver weights increased, hepatocellular pigmentation, cytoplasmatic eosinophilia), kidneys (kidney weights decreased, chronic progressive nephropathy increased), uterus (uterus weights decreased)</p>

Table 14. continued

Tumours:

		Concentration of DEHP in the diet (mg/kg)				
		0 [historical control data]	100	500	1500	6000 6000 recovery
Male mice						
liver						
adenomas	4/70 (6 %)	10/60 (17 %) [#]	13/65 (20 %) [#]	14/65 (22 %) [#]	19/70 (27 %) [#]	3/55 (5 %)
carcinomas	4/70 (6 %)	5/60 (8 %)	9/65 (14 %)	14/65 (22 %) [#]	22/70 (31 %) [#]	12/55 (22 %) [#]
adenomas and carcinomas	8/70 (11 %) [41/149, 27 %]	14/60 (23 %)	21/65 (32 %)*	27/65 (42 %)*	37/70 (53 %)*	14/55 (26 %)*
Female mice						
liver						
adenomas	0/70 (0 %)	2/60 (3 %)	4/65 (6 %)	9/65 (14 %) [#]	34/70 (49 %) [#]	13/55 (24 %) [#]
carcinomas	3/70 (4 %)	2/60 (3 %)	3/65 (5 %)	10/65 (15 %) [#]	16/70 (23 %) [#]	23/55 (42 %) [#]
adenomas and carcinomas	3/70 (4 %) [11/151, 7.3 %]	4/60 (6 %)	7/65 (11 %)	19/65 (29 %)*	44/70 (63 %)*	30/55 (55 %)*

* statistically significantly different from control group; [#] statistically significantly different from control group, MAK calculation

In another study, in which male **Sprague-Dawley rats** were given DEHP with the diet for life, no effects were observed at the low dose of 600 mg/kg diet (30 mg/kg body weight and day). At the middle dose of 1897 mg/kg diet (95 mg/kg body weight and day) the incidence of adenomas and carcinomas of the liver was significantly increased. At the high dose of 6000 mg/kg diet (300 mg/kg body weight and day) in addition the incidence of Leydigomas of the testes was significantly increased; spermiogenesis was also impaired in animals of this dose group (Berger 1995, 1996).

There are also other studies with F344 rats with smaller numbers of animals which do not meet present-day requirements for a carcinogenicity study, but nevertheless confirm the liver tumours in the high dose range. These studies are discussed in IARC (2000) (Cattley *et al.* 1987, Hayashi *et al.* 1994, Rao *et al.* 1987, 1990). Of interest is also a study with Sprague-Dawley rats given DEHP in doses of up to 20000 mg/kg diet for 102 weeks. Liver tumours were not observed in either the control animals or the treated animals. Even if these findings do not exclude carcinogenic effects in this strain, they show that this strain is evidently less sensitive to the carcinogenic effects (Ganning *et al.* 1991).

In a long-term study of the NTP, **B6C3F₁ mice** were exposed to DEHP in doses of 3000 or 6000 mg/kg diet for 103 weeks. Survival, which at the end of the study was over 60 % in the male animals and over 50 % in the female animals, was not found to be

affected by the substance. Body weight gains were reduced in a dose-dependent manner in the female animals. At the end of the study neoplastic changes in the liver were found in both sexes; the incidences for adenomas and carcinomas were significantly increased in both dose groups (NTP 1982).

In another study, groups of 55 to 70 **B6C3F₁** mice of each sex were exposed to doses of DEHP of 100, 500, 1500 or 6000 mg/kg diet for 104 weeks. In addition, a recovery group was exposed to doses of 6000 mg/kg diet for 78 weeks and then put on the control diet for another 26 weeks. In addition to the classical histological evaluation, DEHP-induced cell and peroxisome proliferation was determined at certain intervals. Survival was over 60 % in both sexes in all groups with the exception of significantly reduced survival in the male animals of the highest dose group (merely 30 %). After doses of 500 mg/kg diet or more peroxisome proliferation was also determined at the end of the study. This effect was not observed in the recovery group. After doses of 500 mg/kg diet, the incidence of adenomas and carcinomas of the liver was significantly increased in male animals. A statistically significant increase in liver adenomas relative to the controls was observed after doses as low as 100 mg/kg diet. In the mouse, liver tumours are a spontaneous tumour which occurs more frequently with the increasing age of the animals. The incidence of liver tumours of 14/60 (23 %) observed after doses of 100 mg/kg diet was lower than the historical control incidence for liver tumours in this laboratory of 41/149 (27 %) (3 studies; 1991 to 1996). It is possible that the value for the study control group happened to be lower. Also at this dose there was no correlation with biochemical parameters. This finding is therefore regarded as probably not substance-related. After doses of 1500 mg/kg diet or more, the incidence of adenomas and carcinomas of the liver was significantly increased in female animals. It is noteworthy that in the recovery group the incidence of adenomas was lower than that in the highest dose group (David *et al.* 1999, 2000b).

Groups of 65 to 80 male and female **Syrian hamsters** were exposed in whole animal chambers to concentrations of DEHP of 15 µg/m³ for 24 hours a day, on 5 days a week, until their natural death. Total exposure was to 7 to 10 mg/kg body weight. In another experiment, groups of 50 male and 50 female Syrian hamsters were given intraperitoneal doses of DEHP of 3000 mg/kg body weight once or twice a week or once a month. The total dose was 24000 to 54000 mg/kg body weight. In neither experiment were differences in survival or tumour incidence found between animals exposed to DEHP and control animals (Schmezer *et al.* 1988). As a result of the low exposure concentration in the inhalation experiment and the unusual protocol in the experiment with intraperitoneal administration, the study is not suitable for evaluation of the carcinogenic effects of DEHP.

5.7.2.1 2-Ethylhexanol

Groups of 50 male and 50 female B6C3F₁ mice were given gavage doses of 2-ethylhexanol of 0, 50, 200, or 250 mg/kg body weight for 18 months. Only in female animals was the incidence of liver carcinomas slightly increased in the low and middle dose groups, and significantly increased in the high dose group (controls: 0/50, low dose:

1/50, middle dose: 3/50, high dose 5/50) (see the chapter “2-Ethylhexanol” in Volume 20 of the present series).

Groups of 50 male and 50 female F344 rats were given gavage doses of 2-ethylhexanol of 0, 50, 150, or 500 mg/kg body weight for 104 weeks. In the animals of both sexes, a dose-dependent reduction in body weight gains was observed, and in the female animals of the high dose group increased mortality. The tumour incidence was not increased in any group (see the chapter “2-Ethylhexanol” in Volume 20 of the present series).

6 Manifesto (MAK value/classification)

DEHP induces hepatocellular tumours in the rat and mouse; in the rat, in addition it causes *spongiosis hepatis* and tumours that develop from enterochromaffine cells of the stomach, Leydig cells, and acinar and islet cells of the pancreas. Mononuclear leukaemia is also observed with increased frequency. As a result of the numerous negative results for the genotoxicity of the substance both *in vitro* and *in vivo*, genotoxic effects cannot be involved in the carcinogenic effects of the substance, or only to a small extent. Also the positive results in the initiation–promotion studies and evidence of numerous receptor-mediated and receptor-independent effects resulting from the interaction with feedback mechanisms which control cell proliferation, suggest that mainly tumour-promoting properties are responsible for the formation of tumours. Positive results in cell transformation tests support this. Although the main effects observed – increase in proliferation, activation of proto-oncogenes, activation of Kupffer cells, increase in peroxisomes and mitochondria, inhibition of apoptosis, inhibition of intercellular communication – cannot be combined in a single mechanism, each one can play a decisive role in the formation of tumours. Together, these effects disturb the equilibrium between cell proliferation and cell loss (apoptosis) in the target organ, resulting in an increase in the number of cells. The proliferation stimulus favours either the expansion of cells already initiated or reduces the DNA repair time of genotoxically damaged cells. Low-level exposure to DEHP which does not induce the effects mentioned above is not expected to make a noteworthy contribution to the cancer risk in human. Therefore, DEHP is classified in Carcinogen category 4.

The effects mentioned, such as peroxisome proliferation or inhibition of apoptosis, have been investigated mainly with relatively high doses of DEHP. As, however, histological effects are observed in the testes, kidneys and liver at much lower doses, these are used for the evaluation of a MAK value. Increased vacuolation of Sertoli cells was described in a 13-week study with Sprague-Dawley rats after doses of 500 mg/kg diet (38 mg/kg body weight and day) or more. The NOAEL was found to be 50 mg/kg diet (3.7 mg/kg body weight and day) (Poon *et al.* 1997). In a carcinogenicity study with F344 rats, an increase in the incidence of age-related mineralization of the renal papilla was observed at 100 mg/kg diet (about 6 mg/kg body weight and day). In a 3-generation study only available as an abstract, no dose-dependent effects were found at doses of

300 mg/kg diet (24 mg/kg body weight and day). For B6C3F₁ mice, the NOAEL found in a carcinogenicity study and a multi-generation study was 100 mg/kg diet (20 mg/kg body weight and day). A dose of 50 mg/kg diet, corresponding to 3.7 mg/kg body weight and day, is therefore used as the starting point for the evaluation of a MAK value. For a person with a body weight of 70 kg and an inhaled air volume of 10 m³ in 8 hours, this corresponds to a value of 25.9 mg/m³. Therefore the MAK value of 10 mg/m³ has been retained. As the systemic effects are the most important, DEHP is classified in Peak limitation category II. As a result of the long half-life of 12 hours, an excursion factor of 8 is justified. As only negligibly higher concentrations reach the foetus during peak concentrations, prenatal toxicity is not to be expected even with an excursion factor of 8. Dermal absorption of DEHP is very low. Therefore the substance is not designated with an "H".

A case report of an urticarial reaction to DEHP and only a few positive results in patch tests with DEHP indicate at the most a very slight sensitization potential for DEHP on the skin. Nor do the results from several experimental studies in humans and animals provide evidence of contact sensitization to DEHP. Apart from two case reports of DEHP-induced bronchial asthma of unclear genesis which are difficult to evaluate, there are no useable reports of respiratory sensitization to DEHP, so that DEHP is not designated with either "Sh" or "Sa".

Classification in one of the categories for germ cell mutagens is, on the basis of the data for genotoxicity, not necessary.

DEHP was found to be embryotoxic and teratogenic in the rat and mouse. In Wistar rats, after only prenatal exposure to DEHP, foetal malformation was described after doses of 1000 mg/kg body weight and day or more; the NOAEL is about 200 mg/kg body weight and day (Hellwig *et al.* 1997). In CD-1 mice, malformations were found after doses of 90 and 190 mg/kg body weight and day and above; NOAELs were found of 44 mg/kg body weight and day (Tyl *et al.* 1988), 48 mg/kg body weight and day (NTP 1988) and 75 mg/kg body weight and day (Shiota and Nishimura 1982). As there is a large enough difference to the MAK value of 10 mg/m³ (corresponding to about 1.4 mg/kg body weight and day for exposure at the workplace), Pregnancy risk group C has been retained.

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