Human Health Hazard Assessment

Di-C6-10 alkyl phthalate (Di-C6-10 PE)
(CAS No. 68515-51-5)

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INTRODUCTION

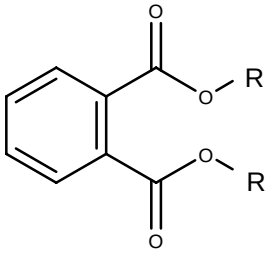
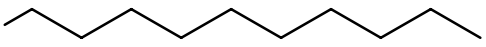
This review of Di-C6-10 alkyl phthalate (Di-C6-10 PE) is a health hazard assessment only. For this assessment, an ECB IUCLID Dataset on Di-C6-10 PE (ECB, 2000) and relevant studies from more recent literature surveys conducted up to September 2006 were consulted. Given Di-C6-10 PE is a mixture of about 1% di-n-hexyl phthalate (DnHP, CAS no. 84-75-3), 20% di-n-octyl phthalate (DnOP, CAS no. 117-84-0), and 79% di-n-decyl phthalate (DnDP, CAS no. 84-77-5), NICNAS hazard assessments for DnHP (NICNAS, 2007a) and DnOP (NICNAS, 2007b) were also considered. NICNAS did not review DnDP and no international reviews were available.

References not marked with an asterisk were examined for the purposes of this assessment. References not examined but quoted from the key reviews as secondary citations are also noted in this assessment and marked with an asterisk. It should be noted that the data in the IUCLID are data reported by the European Chemicals Industry and has not undergone review by the European Commission.

Hazard information from this assessment is published also in the form of a hazard compendium providing a comparative analysis of key toxicity endpoints for 25 phthalates (NICNAS, 2007c).

1. IDENTITY

1.1 Identification of the Substance

CAS Number:	68515-51-5
Chemical Name:	1,2-Benzenedicarboxylic acid, di-C6-10-alkyl esters
Common Name:	Di-C6-10 alkyl phthalate (Di-C6-10 PE)
Molecular Formula:	$C_{27}H_{44}O_4$
Structural Formula:	  (based on C10 length)
	Di-C6-10 PE is a mixture of n-C6, n-C8, and n-C10 phthalates with completely linear alcohol side chains. It contains about 1% DnHP (CAS no. 84-75-3), 20% DnOP (CAS no. 117-84-0), and 79% DnDP (CAS no. 84-77-5) (Jahnke et al., 2005).
Molecular Weight:	434.4 (calculated based on 1% DnHP, 20% DnOP, and 79% DnDP)
Synonyms:	Di(n-hexyl, n-octyl, n-decyl) phthalate; 610P phthalate; Di(C6-C10)alkyl phthalate

Purity/Impurities/Additives: Not available

1.2 Physicochemical Properties

Table 1: Summary of physicochemical properties

Property	*Value*
Physical state	Organic liquid
Melting point	-50°C
Boiling point	250°C (0.5 kPa)
Density	974 – 979 (20°C)
Vapour pressure	$<1 \times 10^{-4}$ kPa (20°C)
Water solubility	$<2 \times 10^{-4}$ g/L (20°C)
Partition coefficient n-octanol/water (log Kow)	>3.5 (20°C)
Henry's law constant	Not available
Flash point	>210°C (open cup)

Source: ECB (2000)

2. USES

Di-C6-10 PE is a primary plasticiser for polyvinyl chloride resins and copolymers. Fifty million pounds of Di-C6-10 PE were produced in the United States in 1994. Di-C6-10 PE is used in PVC utilized in the manufacture of flooring and carpet tile, canvas tarpaulins, swimming pool liners, notebook covers, traffic cones, toys, vinyl gloves, garden hoses, weather stripping, flea collars, and shoes (CMA, 1999*).

Use information in Australia was not available.

3. HUMAN HEALTH HAZARD

3.1 Toxicokinetics

No data

3.2 Acute Toxicity

Study	*Species*	*Results (LD50/LC50)*	*References*
Oral	Rat	>2000 mg/kg bw	Huels AG, 1988*
		>30720 mg/kg bw	Huels AG, 1965*

Source: ECB (2000)

Data not Reported in Previous Evaluations

No data.

Conclusion

Di-C6-10 PE has low acute oral toxicity, with a LD50 for rats of >2000 mg/kg bw. No acute toxicity data from inhalation or dermal exposure or human studies were available for Di-C6-10 PE.

3.3 Irritation

Skin Irritation

In skin irritation tests (Draize and OECD TG 404), Di-C6-10 PE exhibited no to mild skin irritation in rabbits (Scientific Associates, 1975a*; Huels AG, 1989*, cited in ECB, 2000).

Data not Reported in Previous Evaluations

No data.

Conclusion

Di-C6-10 PE caused minimal skin irritation in rabbits.

Eye Irritation

In eye irritation tests (Draize and OECD TG 405), Di-C6-10 PE exhibited slight eye irritation in rabbits (Scientific Associates, 1975b*; Huels AG, 1989*, cited in ECB, 2000).

Data not Reported in Previous Evaluations

No data.

Conclusion

Di-C6-10 PE caused minimal eye irritation in rabbits.

Respiratory Irritation

No data.

3.4 Sensitisation

No data.

3.5 Repeated Dose Toxicity

Data not Reported in Previous Evaluations

In a 21-day study, Di-C6-10 PE at 0, 0.6, 1.2 and 2.5% in the diet (0, 652, 1294, and 2604 mg/kg bw/day in males; 0, 657, 1222, and 2535 mg/kg bw/day in females) was fed to Fischer 344 rats (5/sex/group) (BIBRA, 1985). Treatment did not significantly influence the food intakes or bodyweights of treated animals. In both sexes, absolute and relative liver weights were statistically significantly increased in all treated groups compared to controls. No statistically significant changes were observed in kidney or testes weights.

The majority of male rats had livers that were pale in appearance. In females there was a reduction in the hepatocyte cytoplasmic basophilia in the groups fed 2.5% and in one rat fed 1.2%. This was regarded as an adaptive rather than a toxic effect. In the males the reduction was obscured by extensive lipid deposition in hepatocytes in all treated groups. In histological examination this lipid was seen as vacuolation and was accompanied by slight increases in mitotic activity and cell necrosis. In females, slight necrosis and increased mitotic activity was confined to a few animals from the 1.2 and 2.5% groups. Males at 2.5% had a slight increase in peroxisome numbers and females a moderate increase. There were

statistically significant increases in cyanide-insensitive palmitoyl-CoA oxidation in both sexes at 2.5% and in males at 1.2%. Non-statistically significant increases were also observed in females at 1.2%. Lauric acid 12-hydroxylase activity was increased significantly in both sexes fed 2.5%. The 11-hydroxylase activity was significantly increased in all treated females. These increases in enzyme activity indicate the induction of peroxisome proliferation. Statistically significant but non-dose related reductions in serum cholesterol levels were seen in all female treated groups and the male 0.6% group. At 2.5%, serum triglyceride levels were statistically significantly increased in females and statistically significantly decreased in females. Changes were not dose related in either sex. The NOAEL in this study was not established because significantly increased liver weights and biochemical/histopathological effects in liver were observed at the lowest dose tested (0.6%; 652-657 (males-females) mg/kg bw/day).

In an oral two-generation reproductive study (reported in Section 3.8), Sprague-Dawley rats (24/sex/group) were fed Di-C6-10 PE in the diet at 0, 1000, 3000, and 10000 ppm (approximately 45, 135, and 450 mg/kg bw/day) (Inveresk Research, 1998). Treatment commenced in F_0 animals 10 weeks prior to mating and continued throughout the mating, gestation and lactation period. F_1 animals were weaned on a similar diet to their respective parents and then selected animals were treated for approximately 11 weeks after weaning, prior to mating. Treatment then continued throughout mating, gestation and lactation periods with selected F_2 animals treated until termination.

Reductions in weight gain were reported for F_0, F_1 and F_2 males and reduced food consumptions were reported for F_0 and F_1 males and during lactation in females at 10000 ppm. Liver weights were increased for female rats of all generations at 10000 ppm, for F_0 and F_2 males at 10000 ppm, for F_0 and F_2 rats of both sexes at 3000 ppm and of F_1 females at 1000 ppm. Absolute liver weight was also increased in F_0 females at 1000 ppm but relative liver weight was not statistically significantly different than controls. Mean relative kidney weights of F_0 and F_1 females were increased at 10000 and 3000 ppm and there was a slight increase in relative kidney weights among F_1 females at 1000 ppm. Absolute, but not relative kidney weights in F_1 males (but not other generations) were increased at 10000 and 3000 ppm.

At necropsy, most adults in the F_0, F_1 and F_2 generations at 10000 ppm had gross observations in the liver, including discoloured, enlarged and pale liver with prominent lobulation and/or pale/dark foci. Pale liver foci were also observed for occasional males at 3000 ppm and for occasional females at 10000 ppm in all the generations. Most F_0 and F_1 males at 10000 ppm had histological findings in the liver. Cellular changes included basophilic foci, eosinophilic cell foci, clear cell foci, vacuolation, bile duct hyperplasia and Kupffer-cell pigmentation. No other details were provided. A NOAEL for systemic toxicity was not identified because of increased liver and kidney weights seen among F_1 females at the lowest dose tested of 1000 ppm (45 mg/kg bw/day).

Conclusion

From a 21-day subchronic oral study and a 2-generation reproductive study in different rat species, the liver and kidney appeared to be the main target organs of Di-C6-10 PE. In both studies, absolute and/or relative liver weights were increased. Kidney weights were increased also in the 2-generation study but not the 21-day study. Peroxisome proliferation was observed in the 21-day study.

A NOAEL was not established in either study. The lowest LOAEL was derived from the 2-generation study based on increased liver and kidney weight seen among F_1 females at the lowest dose tested (45 mg/kg bw/day).

3.6 Genetic Toxicity

Di-C6-10 PE was tested in the mouse lymphoma gene mutation assay and considered equivocal due to a non-dose related increase in mutations in the presence and absence of metabolic activation (Barber et al., 2000).

Data not Reported in Previous Evaluations

No data.

Conclusion

Equivocal results for Di-C6-10 PE were obtained in a mouse lymphoma mutation assay. No *in vivo* or human genotoxicity data are available for Di-C6-10 PE.

3.7 Carcinogenicity

Di-C6-10 PE was tested *in vitro* in a mammalian cell transformation assay using Balb/c-3T3 mouse cells. With an exposure period of 72 hours and incubation over 4 weeks Di-C6-10 PE did not induce statistically significant increases in transforming activity with concentrations up to 6.32 µl/mL (Barber et al., 2000).

Data not Reported in Previous Evaluations

No data.

Conclusion

Di-C6-10 PE was inactive in an *in vitro* mouse cell transformation assay. No *in vivo* carcinogenicity data were available for Di-C6-10 PE.

3.8 Reproductive Toxicity

Traditional hazard assessments consider reproductive toxicity separate from developmental toxicity. Reproductive toxicity is tested by exposing sexually mature adults to a chemical and examining the effects on the animal capacity to reproduce. Developmental toxicity is studied by exposing pregnant dams and looking for effects in the foetuses. However, these tests generally do not detect chemicals that induce effects that only appear postnatally. Thus, chemicals that affect the developing reproductive system following prenatal exposure may also affect sexual maturation or functional reproductive disorders that are only apparent at maturity. Developmental toxicity can therefore lead to reproductive toxicity and the two endpoints cannot be clearly distinguished.

In this hazard assessment, data will be presented on the basis of test procedure, including two-generation studies, developmental/prenatal toxicity studies (only the dam is dosed, study ends before parturition) and developmental/postnatal studies (dam is dosed during gestation

and allowed to litter, study ends during weaning). The effects on fertility and development will then be discussed separately in the conclusion.

Data not Reported in Previous Evaluations

3.8.1 Two-generation reproductive toxicity studies

In an oral two-generation reproductive study (Inveresk Research, 1998), Sprague-Dawley rats (24/sex/group) were fed Di-C6-10 PE in the diet at 0, 1000, 3000, and 10000 ppm (0, 45, 135, and 450 mg/kg bw/day). F_0 animals were treated for 10 weeks prior to mating for production of the F_1 litter. Treatment continued throughout the mating, gestation and lactation period until termination after weaning of these litters. F_1 animals were weaned on the same diets as their respective parents. Selected F_1 animals were treated for approximately 11 weeks after weaning, prior to mating. Treatment then continued for both sexes throughout the mating, gestation and lactation periods, until termination at the time of weaning of the F_2 litters. Selected F_2 animals were treated until termination on the completion of the post-weaning assessments. Systemic effects are described in Section 3.5. The LOAEL for systemic effects was 1000 ppm (45 mg/kg bw), the lowest dose tested.

There were no obvious effects of treatment on the mating performance, fertility indices or the duration of gestation. There was no effect on absolute testes weight in treated animals nor seminiferous tubule diameter or qualitative measures of spermatogenesis. However adjusted testes weight of high dose F_1 males was significantly reduced at weaning and significantly increased as adults. The absolute seminal vesicle weights were significantly reduced in F_0, F_1 and F_2 males while the adjusted weights of seminal vesicles in F_1 and F_2 males at 10000 ppm were significantly reduced. There was also a decreased in seminal vesicle weight in F_2 males at 3000 ppm (examined at weaning) but this was considered to be probably incidental in the absence of similar effects in the other generations. Mean prostate weight was reduced among F_1 adults at 10000 ppm.

Developmental toxicity at the highest dose tested (10000 ppm) included decreased litter size and survival PND 4 to 21; decreased pup weights; increased adjusted body weights for F_1 adults; and decreased weaning weights. There was a marginal delay in preputial separation in the F_1 and F_2 generations at 10000 ppm (not significant).

The changes in seminal vesicle weight in the high dose group are considered to be related to body weight decreases due to reduced food intake. The LOAEL for reproductive effects was considered to be 10000 ppm (450 mg/kg bw) based on reduced seminal vesicle weight. A NOAEL for systemic toxicity was not identified due to increased liver and kidney weights among F_1 females at 1000 ppm (45 mg/kg bw), the lowest dose tested. The NOAEL for developmental effects was considered to be 3000 ppm (135 mg/kg bw/day). The LOAEL was 10000 ppm based on slightly decreased litter survival, and slightly decreased pup and litter weight.

Conclusion

Effects on fertility

A two-generation reproductive study indicated that exposure of rats to Di-C-6-10 PE at 1000-10000 ppm in the diet did not induce obvious effects on mating performance, fertility indices

or duration of gestation. The absolute and relative seminal vesicle weight was significantly decreased in association with decreased body weight in the F_1 and F_2 males exposed to high doses of Di-C6-10 PE (Inveresk Research, 1998). Food consumption was also reduced in the high dose F_1 males. However, Chapin et al. (1993) has shown that feed restriction resulting in a 10% reduction in body weight over 15 weeks is associated with decreased prostate and seminal vesicle weight with no effect on testes weight.

The LOAEL for reproductive effects was established at 10000 ppm (450 mg/kg bw), while the NOAEL for systemic toxicity was not identified because increased liver and kidney weights in F_1 females were seen at 1000 ppm, the lowest dose tested.

Effects on development

A two-generation reproductive study for Di-C6-10 PE indicated that there was a marginal, non-significant delay in sexual maturity at 10000 ppm (450 mg/kg bw/day, the highest dose tested), but that sexual maturity at the lower levels was attained at a similar age to that of controls. The NOAEL for developmental effects was established at 3000 ppm (135 mg/kg bw/day), with a LOAEL of 10000 ppm (450 mg/kg bw/d) based on slightly decreased litter survival, and slightly decreased pup and litter weight.

4. HAZARD CHARACTERISATION

Di-C6-10 PE is a mixture containing approximately 1% DnHP, 20% DnOP, and 79% DnDP. Toxicity data for Di-C6-10 PE were not available for the majority of health endpoints. For endpoints with missing or incomplete data, information from structurally similar phthalates, where available, was used to extrapolate potential toxicity. Relevant read-across information was obtained from other NICNAS assessment reports for relevant phthalates and the NICNAS Phthalates Hazard Compendium (2007c) which contains a comparative analysis of toxicity endpoints across 25 phthalates, including Di-C6-10 PE. As DiC6-10 is a mixture, data on the constituents are also considered relevant. NICNAS has completed hazard assessment on two of the constituents, DnHP (NICNAS, 2007a) and DnOP (2007b), but has not assessed DnDP, the predominant constituent.

Limited data on the toxicokinetics were available for DnHP and DnOP and none for Di-C6-10 PE and DnDP. However, based on the toxicokinetic profile of phthalates in general, Di-C6-10 PE is likely to be rapidly absorbed as the monoester from the gut and excreted via the urine.

Di-C6-10 PE has low oral acute toxicity. No acute dermal or inhalation toxicity studies are available for Di-C6-10 PE. Based on data for other phthalates of a similar molecular weight, Di-C6-10 PE is expected to have low acute dermal and inhalation toxicity.

Di-C6-10 PE causes minimal skin and eye irritant effects in rabbits. Although there are no sensitisation studies, Di-C6-10 PE is not expected to cause any skin sensitisation based on data obtained on other phthalates.

In a 21-day repeated dose oral study and a 2-generation reproductive toxicity study with Di-C6-10 PE in different rat species, the liver and kidney appear to be the main target organs. In both studies, absolute and/or relative liver weights were increased. Kidney weights were increased in the 2-generation study but not the 21-day study. Histopathological effects in the

liver include lipid deposition, reduction in the hepatocyte cytoplasmic basophilia, increased mitotic activity and slight cell necrosis. Decreased serum cholesterol levels, slightly increased peroxisome numbers, palmitoyl CoA oxidation and liver metabolising enzymes were also observed in the 21-day study. No NOAEL was established and the LOAEL was 45 mg/kg bw/day.

None of the three constituents of Di-C6-10 PE, DnHP, DnOP and DnDP were genotoxic *in vitro* studies (NICNAS, 2007a & 2007b; CMA, 1999*). Although equivocal results for Di-C6-10 PE were obtained in a mouse lymphoma mutation assay due to a non-dose related increase in mutations in the presence and absence of metabolic activation, it did not induce transformation in Balb/c-3T3 cells. When assessed together, and noting the generally negative genotoxicity profile of phthalates, Di-C6-10 is considered unlikely to be genotoxic.

The only carcinogenicity data available for Di-C6-10 PE is a negative *in vitro* mouse cell transformation assay. There is also limited data for DnOP suggesting it might act as a promoter of preneoplastic lesions in the rat liver, probably by a mechanism not relying on peroxisome proliferation. Overall, due to insufficient testing on phthalates, it is not possible to extrapolate carcinogenic potential for Di-C6-10 PE.

In a two-generation rat reproduction study, Di-C6-10 PE (up to 10000 ppm) in the diet did not induce obvious effects on the mating performance, fertility indices or duration of gestation. The LOAEL for reproductive effects was taken as 10000 ppm (450 mg/kg bw/d), based on reduced seminal vesicle weight, while systemic effects (increased liver and kidney weights in F_1 females) were seen at 1000 ppm (45 mg/kg bw/d), the lowest dose tested.

Di-C6-10 PE caused developmental effects in the same two-generation reproductive study at maternally toxic doses. The NOAEL for developmental effects was established at 3000 ppm (135 mg/kg bw/day), with a LOAEL of 10000 ppm (450 mg/kg bw/d) based on slightly decreased litter survival, and slightly decreased pup and litter weight.

Although the reproductive and developmental effects observed in the two-generation study using Di-C6-10 PE were minor and occurred at dose levels higher that those for systemic toxicity, it should be noted that the constituent DnHP, which is present at 1%, is known to cause reproductive and developmental toxicity. The major constituents DnOP and DnDP are high molecular weight phthalates with backbone carbon lengths of ≥ C7, and are therefore unlikely to induce reproductive and developmental effects.

5. HUMAN HEALTH HAZARD SUMMARY TABLE

Phthalate	***Acute Toxicity***	***Irritation & Sensitisation***	***Repeated Dose Toxicity***	***Genetic Toxicity***	***Carcinogenicity***	***Fertility***	***Developmental Toxicity***
Di-C6-10 alkyl phthalate (Di-C6-10 PE)	Oral Rat: >2000 mg/kg bw/d Dermal No data Inhalation No data	Skin irritation: Minimal effect Eye irritation: Minimal effect Respiratory irritation: No data Skin sensitisation: No data	Rat: NOAEL = not established LOAEL (2-gen. repro study) = 45 mg/kg bw/d, ↑ liver and kidney weights (F1 f) High doses: pale livers with lobulation and discolouration, slight cell necrosis (m). PP noted.	*In vitro* Equivocal in mouse lymphoma assay *In vivo* No data	*In vitro* Negative in cell transformation assay. *In vivo* No data	Rat: NOAEL = 135 mg/kg bw/d LOAEL = 450 mg/kg bw/d, ↓ seminal vesicle weight	Two generation study Rat: NOAEL = 135 mg/kg bw/d LOAEL = 450 mg/kg bw/d, ↓ litter survival, ↓ pup weight

↑: increase; ↓: decrease; m: male; f: female; PP: peroxisome proliferation

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7. ROBUST STUDY SUMMARIES

Repeated Dose Toxicity

Test substance	Di-C6-10 PE
Type of Test	Subchronic Oral Toxicity – Repeat Dose Study
Species	Rats, Fischer 344, 5/sex/group, 34-36 day of age
Route of admin.	Oral
Study duration	21 day
Treatment frequency	Daily
Post exposure period	None
Doses	0, 0.6%, 1.2%, 2.5% (0, 652, 1294, and 2604 mg/kg bw/day in males; 0, 657, 1222, and 2535 mg/kg bw/day in females)
Control group	0% in the diet, and Di-(2-ethylhexyl) phthalate (DEHP) (1.2% or 1200 mg/kg bw) for study comparison
NOAEL / NOEL	Not established
LOAEL / LOEL	652 mg/kg bw (males); 657 mg/kg bw (females)
GLP& QA	Yes
Guidelines	Not known
Method	Di-C6-10 PE at concentrations of 0, 0.6, 1.2 and 2.5% in the diet was fed to Fischer 344 rats (5/sex/group) for 21 days. Another group of animals (5/sex) fed with 1.2% DEHP was used as positive control for induction of liver peroxisome number and palmitoyl CoA oxidation. Bodyweights and food intakes were monitored prior to and throughout the treatment period. The rats were killed after an overnight fast and blood was collected for determination of serum triglyceride and cholesterol levels. The livers, kidneys and testes were weighed and preserved for histological examination. In addition, samples of liver were processed for electron microscopic examination of the peroxisomes, for histochemical demonstration of neutral fat, and for biochemical determination of cyanide-insensitive palmitoyl-CoA oxidation, microsomal lauric acid 11- and 12-hydroxylation, and total and microsomal protein levels.
Result	Di-C6-10 PE treatment did not significantly influence the bodyweights or food intakes of the treated animals. In both sexes the weights and relative weights of the livers were increased in all treated groups. In the females there was a reduction in hepatocyte cytoplasmic basophilia in the group fed 2.5% and in one rat fed 1.2%. In the males the reduction was obscured by extensive lipid deposition in all treated groups. In the histological examination this lipid was seen as vacuolation and was accompanied by slight increases in mitotic activity and cell necrosis. In the females slight necrosis and increased mitotic activity was confined to a few animals from the 1.2 and 2.5% groups. Serum cholesterol levels were significantly reduced in the female treated groups, and the male 0.6% group (not dose-related). Males at 2.5% had a slight increase in peroxisome numbers and females a moderate increase. There were increases of palmitoyl CoA oxidation in both sexes fed 1.2 and 2.5%. Lauric acid 12-hydroxylase activity was increased significantly in both sexes fed 2.5%. The 11-hydroxylase activity was significantly increased in all treated females. DEHP treatment resulted in reduced food intake and lower bodyweight than the controls throughout the study period. In both sexes there was an increase

	in the weight and relative weight of the liver and a reduction in hepatocyte cytoplasmic basophilia. Serum cholesterol levels were also reduced in both sexes. Peroxisome numbers showed a marked (males) or moderate (females) increase after feeding DEHP. Palmitoyl CoA oxidation and lauric acid 11- and 12-hydroxlation were increased in the male and female groups.
Conclusion	Di-C6-10 PE caused a slight (males) or moderate (females) peroxisome proliferation in rats. There were liver weight increase, activation of palmitoyl CoA oxidation and lauric acid 12-hydroxylation. Lauric acid 11-hydroxlation was increased in the females only. In female rats there was a reduction in hepatic lipid levels following administration of Di-C6-10 PE. In male rats there was an extensive fat deposition, with slight increases of mitotic activity and cell necrosis. There was a marked sex difference in this response with the females showing a few instances of increased cell necrosis and mitotic activity and no fat deposition. Given that toxic effects (increased liver weight and histopathologically observed liver cell necrosis and increased miotic activity) were seen at lowest dose tested (0.6%), a NOAEL in this study was not established because significantly increased liver weight and histopathological effects in liver were observed at the lowest dose tested.
Reference	BIBRA (1985) A 21-day feeding study of 610 phthalate to rats: Effects on the liver and liver lipids. Report No. 0495/7/85. The British Industrial Biological Research Association.

Reproductive Toxicity

Test substance	Di-C6-10 PE
Type of test	2-Generation reproductive toxicity
Species	Rats, Sprague-Dawley, 24/sex/group, 4-week of age
Route of admin.	Oral
Study duration	2-generation. Treatment from 10 weeks prior to mating in F_1 and continuously to F_1 until termination on the completion of the post-weaning assessment in F_2 animals (the number of days after weaning is not specified for F_2).
Treatment frequency	Daily
Post exposure period	None
Doses	0, 1000, 3000, 10000 ppm (~0, 45, 135, 450 mg/kg bw/day)
Control group	24/sex
NOAEL / NOEL	not established for systemic toxicity; 135 for fertility and development
LOAEL / LOEL	45 mg/kg bw/d for systemic toxicity; 450 mg/kg bw/d for fertility and developmental toxicity
GLP& QA	Not known
Guidelines	Not known
Method	Sprague-Dawley rats (24/sex/group) were fed Di-C6-10 PE in the diet at 0, 1000, 3000, and 10000 ppm. F_0 animals were treated for 10 weeks prior to mating and throughout the mating, gestation and lactation period until termination after weaning of these litters. F_1 animals were weaned on the same diets as were fed to their respective patents. The selected F_1 animals were treated for 11 weeks after weaning, prior to mating. Treatment then continued for both sexes throughout the mating, gestation and lactation periods, until termination at the time of waning of the F_2 litters. Selected F_2 animals were treated until termination on the completion of the post-weaning assessments. Food consumption and body weight was recorded weekly. Females were housed with males for a maximum of 7 nights and allowed to litter normally. Pups were examined on postnatal days 0-4, 7, 14 and 21. At weaning, pups were selected for mating and post-weaning evaluation. At necroscopy, reproductive organs, liver, kidney and pituitary were weighed. Only high dose and control groups were assessed histologically.
Result	Reductions in weight gain in F_0, F_1 and F_2 males and reduced food consumptions in the F_0 and F_1 males, and some reduction in food consumption during lactation in females at 10000 ppm were reported. Mean kidney weights of F_0 and F_1 females, and F_1 males were increased at ≥3000 ppm. There was a slight increase in kidney weights among F_1 females at 1000 ppm. Liver weights of F_0 and F_2 in both sexes at ≥3000 ppm and of F_1 females at 1000 ppm were increased. Absolute liver weight was also increased in F_0 females at 1000 ppm but relative liver weight was not statistically significantly different than controls. At necropsy, most adults in the F_0, F_1 and F_2 generations at 10000 ppm had gross observations in the liver, including discoloured, enlarged and pale liver with prominent lobulation and/or pale/dark foci. Pale liver foci were also observed for occasional males at 3000 ppm and for occasional females at 10 000 ppm in all the generations. Most F_0 and F_1 males at 10 000 ppm had histological findings in the liver; cellular changes included basophilic foci, eosinophilic cell foci, clear cell foci, vacuolation, bile duct hyperplasia and Kupffer-cell pigmentation. There were no obvious effects of treatment on the mating performance, fertility indices or the duration of gestation. There was no effect on testes weight in treated animals nor seminiferous tubule diameter or qualitative measures of spermatogenesis. The weights of seminal vesicles in F_1 and F_2 adults at 10000 ppm were reduced, and this reduction extended to F_2 adults at 3000 ppm. Mean

	prostate weight was reduced among F_1 adults at 10000 ppm. Developmental toxicity included that at highest dose tested (10000 ppm) decreased litter size and survival across day 4 to 21; decreased pup weights; increased adjusted body weights for F_1 adults; and decreased weaning weights. The weights of seminal vesicles were reduced in F_1 adults at 10000 ppm and F_2 adults at 3000 ppm. Mean prostate weight was reduced among F_1 adults at 10000 ppm. There was a marginal delay in sexual maturity at 10000 ppm.
Conclusion	The NOAEL for fertility and developmental effects was considered to be 3000 ppm (135 mg/kg bw/day), with a LOAEL of 10000 ppm based on slightly decreased litter survival, and slightly decreased pup and litter weight. The NOAEL for systemic toxicity was not identified because increased liver and kidney weights among F_1 females were seen at 1000 ppm (45 mg/kg/bw/day), the lowest dose tested.
Reference	Inveresk Research (1998) Witamol 110/Liplast 610P – Two generation reproduction study in rats. Report No. 15380, Doc ID: 88980000172. Scotland, UK, Inveresk Research (Unpublished report submitted by Condea Vista Co.).