OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

DIETHYL PHTHALATE

adopted by the SCCNFP during the 20th Plenary meeting of 4 June 2002

1. Terms of Reference

1.1 Context of the question

Diethyl phthalate is used in a wide range of consumer goods. It has been the subject of great public concern in recent years. The Commission has received notification of extensive uses of this material and issues regarding its safety have been raised.

Diethyl phthalate is currently used through direct addition in cosmetic products and indirectly in fragrances. The material is listed in the inventory of ingredients employed as a solvent and vehicle in fragrance and cosmetic products, as well as a denaturant, and film former.

1.2 Request to the SCCNFP

The SCCNFP has been requested to review the safety of diethyl phthalate and to answer the following questions:

* Does the safety profile of Diethyl phthalate support its use in cosmetic products at current levels?
* Should restrictions on the concentration or fields of exposure be placed upon the use of this material in cosmetic products?

Whilst it does not fall under the competence of cosmetic products, it may also be appropriate for the SCCNFP to consider the use of this material in other non-food consumer products.

1.3. Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary name

Diethyl phthalate (INCI name)

2.1.2. Chemical names

1,2-benzenedicarboxylic acid, diethyl ester; diethyl *o*-phthalate; ethyl phthalate; *o*-benzenedicarboxylic acid diethyl ester; diethyl ester phthalic acid; diethyl-*o*-phenylenediacetate.

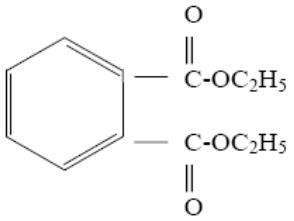
2.1.3. Trade names and abbreviations

Anozol, DPX-F5384, Estol 1550, Neantine, Palatinol A, Phthalol, Placidol E, Solvanol, Unimoll DA.
Abbreviations: DEP

2.1.4. CAS no.

84-66-2

2.1.5. Structural formula



2.1.6. Empirical formula

Empirical formula: $C_{12}H_{14}O_4$
Mol. Weight: 222.26

2.1.7. Purity, composition and substance codes

The purity of manufactured phthalate ester is reported between 99.70% and 99.97%, with the main impurities being isophthalic acid, terephthalic acid, and maleic anhydride.

2.1.8. Physical properties

Subst. Code:	diethyl phthalate
Appearance:	clear, colourless, practically odourless liquid
Melting point:	- 40.5°C
Boiling point:	298°C (295-302°C)
Flash point:	161°C
Density:	1.120 g/ml at 25°C
Vapour Pressure:	< 0.001 torr at 20°C
Log $P_{ow:}$	2.47 (1.4 – 3.3)
Henry's law constant:	7.8 x 10^{-7} atm m³/mol

2.1.9. Solubility

Water: fairly soluble, 1.08 g/l
Miscible in all quantities in alcohol, ether, acetone, ketones, benzene, esters, aromatic hydrocarbons, aliphatic solvents and vegetable oils.

2.2. Function and uses

Diethylphthalate is used as solvent and vehicle for fragrance and cosmetic ingredients, as well as alcohol denaturant. DEP was reported in 1995 as an ingredient in 67 formulations in the USA at concentrations ranging from less than 0.1% to 50%.
They include bath preparations (oils, tablets, salts), eye-shadows, toilet waters, perfumes and other fragrance preparations, hair sprays, wave sets, nail polish, and enamel removers, nail extenders, nail polish, bath soaps, detergents, after-shave lotions, and skin care preparations.
As an illustration a table summarising the most common uses in cosmetics and perfumes is given below.

Most common uses of DEP in cosmetics and perfumes

Product category	Total n° of formulations in category	Total n° containing Ingredient	No. of Product Formulations within each Concentration Range (%)				
			> 25-50	> 10-25	> 5-10	> 1-5	> 0.1-1
Bath oils, tablets and salts	237	3				1	
Other bath preparations	132	2					
Eye shadow	2582	1					
Colognes and toilet waters	1120	19				1	10
Perfumes	657	23	1			1	7
Fragrance powders (dusting and talcum)	483	1					1
Sachets	119	3				1	2
Other fragrance prep.	191	2	1				1
Hair sprays (aerosol fixatives)	265	5				2	3
Wave sets	180	1					1
Nail polish and enamel remover	41	1				1	
Bath soaps and detergents	148	1					1

Product category	Total n° of formulations in category	Total n° containing Ingredient	No. of Product Formulations within each Concentration Range (%)				
			> 25-50	> 10-25	> 5-10	> 1-5	> 0.1-1
Aftershave lotions	282	3					3
Face, body, and hand skin care preparations (excluding shaving preparations)	832	1					
Other skin care preparations	349	1					1
1981 Total		67	2			7	30

Another source (1995) gives a range of concentration of DEP in 5 different after-shave lotions between 0.05% to 10.1%.

Otherwise, Diethyl phthalate is used as a plasticizer for cellulose ester plastic films and sheets (photographic, blister packaging, and tape applications) and molded and extruded articles (consumer articles such as toothbrushes, automotive components, tool handles, and toys).

Ref.: 4, 13, 19, 20, 21, 74,116

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

DEP has a low order of acute oral toxicity.
LD 50 are reported, from the public literature, in the range of 1 to 31 g/kg by oral route in mice, rats, rabbits dogs, and guinea pigs.

Oral LD50 in various species (g/kg) according to the study reported					
Species	**Mouse**	**Rat**	**Guinea pig**	**Rabbit**	**Dog**
	6.2	9.2a	8.6	1.0	5.0
		9.4	> 4.0		
		> 5.6b			
		9.5 - 31			
		8.6			

a LD50 is equal to 8.2 ml/kg corresponding to 9.2 g/kg
b LD50 is higher than 5 ml/kg corresponding to higher than 5.6 g/kg

Clinical signs have included CNS depression, convulsion and respiratory paralysis prior to death. In humans an LDLo (lower lethal dose) of 0.5 g/kg has been reported.

Ref.: 4, 7, 8, 13, 19, 48, 58, 68, 69, 70, 74, 77, 85, 86, 90, 104

2.3.2. Acute dermal toxicity

Rat
DEP has a low acute dermal toxicity, higher than 10 ml/kg corresponding to 11 g/kg. Doses of application were 1, 2, 5 and 10 ml/kg. The rats (3 males and 3 females) received a single application of the test substance dermally under an occluded patch. No deaths were reported at any dose. Slight redness of the skin at the site of application was observed at 24 hr for all concentrations, but not further. No other clinical signs have been reported. The bodyweight gain of the animals during the 14 day observation period was normal. No gross changes were noted on animals sacrificed at the end of the treatment period. An LD50 of 3 g/kg in Guinea pig was also reported.

Ref.: 20, 86

2.3.3. Acute inhalation toxicity

The following results have been reported:

LC50 after inhalation in rats: 7.5 g/m^3
LC50 after inhalation in mice: 4.9 g/m^3
In humans, LC50: 1.0 g/m^3

Ref.: 20, 70

2.3.4. Repeated dose oral toxicity (up to two weeks)

Rat
Young male Wistar rats received with the diet a concentration of 2% DEP (98% purity) (approximately 1000 mg/kg/day) for seven days. They reported an increased absolute and relative liver weight ($p < 0.05$) and a significant decrease of testosterone concentrations in testes and serum. Apart from a reduced bodyweight gain on the first day of treatment there were no effects on body weight, kidney and testes weights, liver, kidney, and in zinc concentration in testes, or dihydro-testosterone concentration in serum.

Remark
Zinc concentrations in testes as well as dihydro-testosterone level were checked with-regard to results published showing variations in a few other phthalate esters.

Mouse
During a 14 day study (Preliminary study to a developmental one) DEP (>99% purity), has been given to male and female 8 weeks old CD-1 mice (8 males and 8 females per dose) at concentrations of 0.25. 0.50, 1.0, 2.5 or 5.0% (approximately 500, 1000, 2000, 5000 or 10000 mg/kg/day. No deaths, signs of toxicity or significant effects on body weight were reported.

Ref.: 68, 72

2.3.5. Repeated dose dermal toxicity (up to 2 weeks)

Rat
In a 2-week dermal study (GLP compliant), undiluted DEP (no purity level given) was applied to male and female Sprague-Dawley rat (5 males and 5 females per group), at the doses of 0 (controls) and 2 ml/kg/day, under a 6-hour semi-occlusive patch.
No changes were observed in body weight gain, clinical chemistry and haematology parameters. Irritation of the skin was evident at the test site, showing erythema and/or slight desquamation. At histological examination a very mild epidermal thickening and a slight hyper-keratosis were noted, with no other histological changes.

Ref.: 95

2.3.6. Sub-chronic oral toxicity

Rat
Diets containing 0 (controls), 0.2, 1 or 5% DEP (approximately 0, 150, 770 or 3160 mg/kg/day for males and 0, 150, 750 or 3710 mg/kg/day for females) were given to male and female Sprague-Dawley rats for 16 weeks. 15 rats of each sex were assigned to each group. DEP specifications were given, level of purity min. 99%. Additional groups of 5 males and 5 females were fed similar diets for 2 or 6 week, mainly for haematology examination and urinalysis. At the end of the appropriate feeding period blood and serum were taken for analysis. Then the rats were deprived of food overnight and subjected to a full macroscopical and microscopical examinations. 12 organs per animal were removed and weighed. The same organs plus 18 others were preserved for histo-pathological examination. Rats showed no changes in behavioural pattern, no deaths occurred.
Reduced food intake and body weight gain were noted in females fed 1% and 5% DEP and in males fed 5% DEP. No statistically significant effects were noted on water intake (see table below).

Mean bodyweight and food and water consumption of rats fed DEP at 0-5% in the diet for 16 weeks										
Dietary level (%)	**Body weight (g) at day**				**Food consumption (g/rat/day) at day**				**Mean food consumption (g/rat/day)**	**Mean water intake (g/rat/day) up to day 112**
	0	27	56	112	1	27	56	112		
Males										
0	125	356	473	599	15.5	27.3	31.3	21.5	24.9	35.2
0.2	125	352	476	617	17.3	29.7	29.5	21.7	25.3	33.4
1.0	124	334*	459	575	13.8	30.0	25.9	21.8	24.7	31.7
5.0	124	271*	356***	461***	3.2*	25.5	23.8	20.2	19.1*	34.4
Females										
0	109	225	283	358	13.7	18.5	21.9	15.2	18.5	31.9
0.2	109	235	286	347	13.9	19.3	17.1	15.8	17.7	27.1
1.0	109	213*	266*	328*	11.4	16.2	19.3	14.7	16.5*	35.5
5.0	108	191***	234***	285***	3.7*	16.6	16.1	14.3	15.1*	28.1

0 = first day of feeding.
Bodyweights are means for groups of 15 animals, and food and water consumption figures are means for three cages each of five rats. Values marked with asterisks differ significantly (Student's *t* test for bodyweights and ranking method of Wilcoxon (1945) for food consumption from those of the controls: *$p < 0.05$; ***$p < 0.001$

Apart from a few variations noted, the results of haematological examinations, urinary cell-excretion rate, renal concentration tests or histological examinations were normal. Serum analysis performed at 16 week was reported as giving normal results, no figures were reported.

There was an increase in relative weights of several organs in both sexes, primarily at the highest dose (brain, kidney, liver, stomach, small intestine and caecum).
The most consistent significant finding was an increase of the relative liver weight in females in all treatment levels, though moderate at 0.2% concentration (see table below).

Relative organ weights of rats fed 0 - 5% DEP in the diet for 2, 6 or 16 wk									
		Relative organ weights (g/100g body weight)							
Sex and dietary level (%)	No. of rats	Brain	Liver	Kidneys	Stomach	Small intestine	Caecum		Terminal body weight (g)
							Empty	full	
Wk 2									
Male									
0	5	0.97	3.37	0.93	0.55	3.66	0.42	1.79	194
1.0	5	0.99	3.67*	0.99	0.59	3.67	0.46	1.51	193
5.0	5	1.23*	4 78***	1.04*	0.84**	3.97	0.53	2.24	149***
Female									
0	5	1.15	3.63	1.00	0.83	3.35	0.44	1.71	157
1.0	5	1.20	3.56	0.99	0.64	3.29	0.48	1.65	149
5.0	5	1.31*	481***	1.06	0.86	3.79**	0.49	2.14	134***
Wk 6									
Male									
0	5	0.55	2.56	0.76	0.39	1.89	0.27	0.74	398
1.0	5	0.55	2.94*	0.75	0.40	2.10	0.30	0.77	385
5.0	5	0.76**	3.41**	0.81	0.52*	2.57***	0.36*	1.15*	274***
Female									
0	5	0.80	2.69	0.74	0.46	2.76	0.40	1.01	237
1.0	5	0.84	2.93	0.70	0.48	2.58	0.45	1.34	231
5.0	5	0.97**	3.57***	0.81	0.59***	2.78	0.42	1.50**	199**
Wk 16									
Male									
0	15	0.39	2.22	0.57	0.29	1.54	0.21	0.60	568
0.2	15	0.39	2.16	0.58	0.29	1.53	0.23	0.60	585
1.0	15	0.39	2.29	0.57	0.32*	1.57	0.24	0.71	559
5.0	15	0.50**	2.95***	0.67***	0.41***	1.93***	0.24	0.89**	438***
Female									
0	15	0.65	2.17	0.62	0.35	1.99	0.27	0.77	330
0.2	15	0.64	2.31*	0.62	0.40***	2.23**	0.30*	0.86	328
1.0	15	0.68	2.35**	0.64	0.41***	2.26*	0.29	0.89	304*
5.0	15	0.78***	2.84***	0.69***	0.51***	2.47***	0.34***	1.43***	267***

Values are the means for the numbers of rats shown and those marked with asterisks differ significantly (Studen'*t* test) from the controls values: *p < 0.05; **p < 0.01; ***p < 0.001.

The relative organ weights increase is, at least, linked to the reduced body weight gain observed. For the liver, in the absence of true abnormal histological findings, the increase of relative weight might be due to hypertrophy linked to an enzyme induction produced by peroxisome proliferation well known with the phthalate esters.
The significance of increased kidney weight at the 5% dietary level, without changes at histo-pathological examination is vague, though such effect had been already noted for several other phthalate esters.
The enlarged liver noted at 1 and 5% concentration represents a moderate adverse response to DEP.

In summary, in rat, toxic signs after 16 weeks of exposure to DEP in the diet consisted of an increase in relative liver weight (without significant abnormal histological findings) in female rats at concentrations at 1 and 5% concentrations and in a less extent at 0.2% (corresponding to 150 mg/kg/day).

An increase in relative weight of some other organs (brain, kidneys, stomach, small intestine and

caecum) in male and or female rats was also noted at the two highest concentrations (corresponding to 750 and 3710 mg/kg/day).

At 0.2% concentration, the increase in the relative liver weight in females is slight and the significance is at $p < 0.05$.
In the absence of a clear liver toxicity after long-term studies in mice and rats (see paragraph 2.9) we can establish the NOAEL as follows:

NOAEL = 0.2% concentration in the diet (corresponding to 150 mg/kg/day).

The microsomal carboxylesterase activities in tissue of Clofibrate-fed mice and rats are altered by DEP confirming it as a peroxisome proliferator.

Ref.: 3, 10

2.3.7. Sub-chronic dermal toxicity

Mouse
In a 4-week study, groups of 10 male and 10 female $B6C3F_1$ mice received dermally DEP, at volumes of 0, 12.5, 25, 50 or 100 µl (0, 15, 31, 62 and 123 µg/kg), applied neat, 5 days/week. 100 µl can be considered as a reasonable maximum volume for mouse studies involving daily skin application.
Increased absolute and relative liver weights were observed only in females receiving 25 and 100 µl DEP. No other adverse clinical signs of toxicity (no death, as well as no effect on bodyweight gain and food consumption) or dermato-toxicity were reported. No histological findings were reported.

Rat
In a 4-week study groups of 10 males and 10 females F344/N rats were given by dermal applications volumes of 0, 37.5, 75, 150 and 300 µl (0, 46, 92, 184 and 369 µg) applied neat, 5 days/week. 300 µl can be considered as a reasonable maximum volume for rat studies involving daily skin application.
There were no clinical signs of toxicity (no death in the treated groups, no clear effects on weight gain or food consumption), and no dermal-toxicity.
An increase in the relative liver weights was noted in 300 µl males and in 150 and 300 µl females. Increased relative kidney weights were seen in 150 and 300 µl males and in 150 µl females.
No other adverse effects were observed in this study.
Signs of toxicity in rats and mice after 4 weeks of dermal exposure to undiluted DEP were limited to increases in weights of liver and kidneys at the two highest doses of each species. The purpose of these studies was to fix the doses to be applied in a long-term study in both species by dermal route.
The methodology was for that reason simplified; no clinical pathological tests were performed. Thus no NOEL or NOAEL can be established

Ref.: 70

2.3.8. Chronic toxicity (see paragraph 2.9)

Specific validated chronic toxicity studies have not been reported. Chronic effects have been reported in 2-year feeding study in mice and rats (see paragraph 2.9).

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

In animal

Assay 1
Substance: DEP (purity not given)
Species: albino rabbits
Dose: 0.5 ml
Concentration: neat
Application: once, 4 h

Number of animals	Skin	Area	Nature of application	Duration of application	Skin reading
4	flank	2.5 x 2.5 cm	Semi-occluded	4 h	1, 24, 48 h

Result: Very slight irritation

Assay 2
Substance: DEP (purity 98-99%)
Species: albino rabbits
Dose: not given
Concentration: neat
Application: repeated 25 days

Number of animals	Skin	Area	Nature of application	Duration of application	Skin reading
3	abdomen	3 x 3 inch	occluded	repeated, 25 applications	Not given

Result: Very slight irritation.

Assay 3
Substance: DEP (purity not given)
Species: albino rabbits
Dose: 0.5 ml
Concentration: neat
Application: once, 24 h

Number of animals	Skin	Area	Nature of application	Duration of application	Skin reading
5	back	1 x 1 inch	Occluded, intact and abraded skin	24 h	24, 72 h

Result: not irritant.

Assay 4
Substance: DEP (purity not given)
Species: albino rabbits
Dose: 0.5 ml
Concentration: neat
Application: 5 days per week for 4 weeks

Number of animals	Skin	Area	Nature of application	Duration of application	Skin reading
3	back	6 cm^2	Semi-occlusive	4 h	1, 24, 48, 72, 168 h

Result: not irritant

Assay 5
Substance: DEP (purity > 99%)
Species: rat
Dose: 0, 37.5, 75, 150, 300 µl
Concentration: neat
Application: 5 days per week for 4 weeks

Number of animals	Skin	Area	Nature of application	Duration of application	Skin reading
20				4 weeks	

Result: not irritant

Assay 6
Substance: DEP (purity > 99%)
Species: mouse
Dose: 0, 12.5, 25, 50, 100 µl
Concentration: neat
Application: 5 days per week for 4 weeks

Number of animals	Skin	Area	Nature of application	Duration of application	Skin reading
20				4 weeks	

Result: not irritant

Assay 7
Substance: DEP (purity > 99%)
Species: rat
Dose: 0, 100, 300 µl
Concentration: neat

Application: 5 days per week for 103 weeks

Number of animals	Skin	Area	Nature of application	Duration of application	Skin reading
120				103 weeks	

Result: not irritant

Assay 8
Substance: DEP (purity 99%)
Species: mouse
Dose: 0, 7.5, 30 µl
Concentration: neat
Application: 5 days per week for 103 weeks

Number of animals	Skin	Area	Nature of application	Duration of application	Skin reading
120				103 weeks	

Result: not irritant

<u>In humans</u>

Assay 1
Substance: DEP (purity not given)
Species: 231 human volunteers
Method: Patch test 24-48 h
Application: closed
Concentration: neat
Reading: 30 minutes

Result: erythema in 4 subjects, considered as non-irritant

Assay 2
Substance: DEP (purity not given)
Species: 46 human volunteers
Method: modified repeated insult patch test (three applications only)
Concentration: neat

Result: not irritant

Assay 3
Substance: DEP (purity not given)
Species: 44 human volunteers
Method: repeated insult patch test
Conc. Induction: neat

Conc. Challenge: neat

Result: no irritation, no sensitization

Assay 4
Substance: DEP (purity not given)
Species: 10 human volunteers
Method: modified repeated insult patch test, 10 days (Kligman-Wooding Test)
Conc. Induction: neat
Conc. Challenge: neat

Result: not irritant

Ref.: 70, 80, 81, 82, 84, 92, 93, 94, 108

2.4.2. Irritation (mucous membranes)

Assay 1
Substance: DEP (purity > 96%)
Species: albino rabbits
Dose: 0.1 ml
Concentration: neat
Application: single

Result: irritation at 1 hour that decreased at 24 hours.

Assay 2
Substance: DEP (purity not given)
Species: albino rabbits
Dose: 0.1 ml
Concentration: neat
Application: single

Result: no irritant.

Assay 3
Substance: DEP (purity not given)
Species: albino rabbits
Dose: 0.1 ml
Concentration: 12.5% in 98% ethanol
Application: single

Result
No corneal opacity or iris congestion, but severe conjunctival irritation including chemosis and discharge. On the seventh day, irritation disappeared, but slight vessel injection was still present.

Assay 4

Substance: DEP (purity not given)
Species: albino rabbits
Dose: 0.1 ml
Concentration: neat
Application: single

Result: slight irritation at 1 hr. to three days, no changes after four days.
Ref.: 17, 47, 58, 78, 79, 87

2.5. Sensitisation and photo-sensitisation

2.5.1. Sensitisation

Assay 1
Substance: DEP (purity not given)
Species: guinea pigs, number of animals not given
Method: maximisation test, open epicutaneous test, Draize test, Freund's Complete adjuvant test
Conc. Induction: neat
Conc. Challenge: neat

Result: no sensitisation

Assay 2
Substance: DEP (purity not given)
Species: guinea pigs, 6-8 animals per group
Method: open epicutaneous test
Conc. Induction: 10.0 %
Conc. Challenge: 10.0 %

Result: no sensitisation

Assay 3
Substance: DEP (purity not given)
Species: 12 guinea pigs
Method: Buehler test
Conc. Induction: neat
Conc. Challenge: neat

Result: no sensitisation

Assay 4
Substance: DEP (purity not given)
Species: 25 human volunteers
Method: human maximisation test
Conc. Induction: 10%
Conc. challenge: 10%

Result: no sensitization

Assay 5
Substance: DEP (purity not given)
Species: 30 human volunteers
Method: human maximisation test
Conc. Induction: neat
Conc. Challenge: neat

Result: no irritation, no sensitization

Assay 6
Substance: DEP (purity not given)
Species: 32 human volunteers
Method: Photo-maximisation test (Kaidbey &Kligman)
Conc. Induction: 25% DEP in ethanol
Conc. Challenge: 25% DEP in ethanol

Result: no sensitisation or photo-sensitisation

Assay 7
Substance: DEP (purity not given)
Species: 32 human volunteers
Method: Photo-maximisation test (Kaidbey &Kligman)
Conc. Induction: 25% DEP in ethanol
Conc. Challenge: 25% DEP in ethanol

Result: no sensitisation or photo-sensitisation

Ref.: 11, 34, 51, 52, 88, 89, 96, 97

2.5.2. Photo-sensitisation

In the last two studies summarised above, no-photosensitization was observed.

Ref.: 96, 97

Human data
No positive patch test reactions were observed in a case of exacerbation of psoriasis due to cosmetics, in a case of systemic contact dermatitis to tea tree oil, in a case of contact dermatitis to a jogging cream, two cases of contact dermatitis in dental technicians, two cases of airborne pigmented contact dermatitis to musk ambrette. A positive reaction to DEP was found in a patient with spectacle frame contact dermatitis.

In 11 patients with occupational dermatitis to acrylates, and in 51 patients tested with plastic and glue allergens, no sensitisation to DEP was detected by patch testing. In 20 patients with perfume dermatitis, no sensitisation to DEP was detected. In 28 patients with perfume dermatitis, no sensitisation to DEP was detected. In 79 cases of eyelid dermatitis, no sensitisation to DEP was

detected. In 310 patients routinely tested for contact dermatitis, no case of DEP sensitisation was observed. In 1532 cases of contact dermatitis tested with a phthalate mix (6% in petroleum), only 1 positive reaction was reported. In 16 patients with spectacle frame dermatitis, one patient reacted to DEP. Out of 60 workers in a factory producing shoes from polyvinyl-chloride tested with DEP, 2 reacted.
Patch tests performed in more than 1000 patients with 48 frequently used constituents of perfumes, DEP being the vehicle, did not show any positive reactions.

Ref.: 14, 15, 16, 23, 38, 35, 40, 56, 57, 61, 66, 73, 76, 102, 106, 111, 117

Conclusion
Diethyl phthalate is a rare sensitiser, and not a photo-sensitiser.

2.5.3. Phototoxicity

In humans

Twenty-nine human volunteers received a single 24h-application of duplicate patches on naïve sites. One of the duplicate patch sites was exposed to UVB and UVA radiation for evaluation of phototoxic potential while the other site was used to evaluate primary irritation potential or to serve as a non-irradiated control. As usual the tests articles utilised were coded.
Three groups were constituted: Group A (positive), 1% w/w dimethyl anthranilate in 25% v/v DEP in ethanol, group B 25% v/v DEP in ethanol, group C blank patch.
Under the conditions of this study, the test substance did not induce any phototoxic reaction.
Due to some abnormal reactions noted among the groups of the first study, a second one has been performed following the same protocol.
Group A (positive control, 1% w/w dimethyl anthranilate in 25% v/v DEP in ethanol) showed evidence of photo-toxicity. Group B (25% v/v DEP in ethanol) showed evidence of mild irritant effect, and Group C (control) did not show evidence of photo-toxicity in the thirty-five human subjects who completed the study.

Ref.: 98, 99

2.6. Reproduction Toxicity

Embryotoxicity by dermal route in mice

DEP was administered cutaneously to pregnant Jcl:ICR mice, (groups 17 to 20 females), in daily doses of 500, 1600 and 5600 mg/kg/day from day 0 through day 17 of gestation and foetuses were removed by caesarean section on day 18.
DEP produced abnormal behaviour possibly caused by pain in mice in a dose dependent manner. Maternal toxicity was evidenced at all doses by reduced thymus and spleen weights and at the high dose by increased adrenal weight. Foetal body weight was reduced significantly at the high dose and skeletal examinations showed a higher incidence of cervical and lumbar rib variations at the high dose. However, no external, visceral or skeletal anomalies in the foetuses were attributable to DEP treatment. The authors concluded that DEP has no potential to produce teratogenic effects on foetuses under these conditions.

NOAEL for maternal toxicity = < 500 mg/kg
NOAEL for foetal toxicity = 1600 mg/kg

Embryotoxicity by oral route in rats

Dietary concentrations of DEP at 0.25%, 2.5% or 5% were administered to timed-pregnant CD rats on gestation days 6 through 15; the rats were sacrificed on gestation day 20. The average nominal doses based on food consumption of controls were 200, 2000 and 4000 mg/kg/day. The actual average doses because of decreased food consumption were approximately 200, 1900 and 3300 mg/kg/day. The study was performed in replicate; the total treated animals reached 31 in controls, and 32 in each of dosed animals.

Maternal toxicity was shown by decreased food consumption, decreased body-weight gain and decreased water consumption, being statistically significant at the highest dose. Gravid uterine weight, absolute and relative maternal liver and kidney weights were unaffected by DEP treatment. No adverse effect on embryo/foetal growth, viability or incidence of external, visceral or skeletal malformations was observed. An increased incidence of one extra rib in the offspring from rats in the maternally toxic high dose group was seen.

NOAEL for maternal toxicity	**=**	**0.25% DEP equal to 200 mg/kg**
NOAEL for foetal toxicity	**=**	**5% DEP equal to 4000 mg/kg**

Conclusion

DEP administered to pregnant rats during the period of major organo-genesis had no adverse effect upon embryo/foetal development.

The only effect noted was an increase in the incidence of extra rib (a commonly observed variation) at a maternally toxic exposure level.

Ref.: 24, 69, 75, 109

2.6.1. One-generation reproduction toxicity

Oral route mice

Male and female CD-1 mice were given DEP at concentrations of 0.25, 1.25 or 2.5% in the diet, before, during and after cohabitation in a continuous breeding protocol. The corresponding doses in mg/kg were 460, 2440 or 4400. The study was GLP compliant.

Continuous exposure of mice (11 weeks of age at outset) to these dose levels of DEP during the 7-day pre-mating, 98-day cohabitation, and 21-day segregation periods had no effect on the number of pairs able to produce at least one litter. There was no effect on the number of litters produced per pair, proportion of pups born alive, sex of pups born alive and live pup weight. The low and mid-dose groups actually showed more live pups per litter compared with the control and high dose group.

The fertility and reproductive performance was assessed along a F1 generation, for the control and high dose groups. The treated group showed a reduction in body weight gain, decreased litter size (when sexes were combined, but not when analysed separately), decreased sperm concentration (no change in sperm motility or percentage of abnormal sperm), increased prostate weight, increased liver weight in females, and reduced uterus and pituitary weight. There were no statistically significant effects on mating behaviour, proportion of pups born alive, live pup weight or sex of pups born alive.

In summary, effects on general reproductive performance with DEP were limited only at the F1 generation to changes at a dose causing decreased body weight gain.

NOEL F0 generation	**> 2.5 % DEP**	**equivalent to**	**> 4400 mg/kg**
NOAEL F1 generation	**< 2.5 % DEP**	**equivalent to**	**< 4400 mg/kg**

Ref.: 55, 65, 68, 75

Male reproductive function specific studies
Special attention has been paid to male reproductive system, on the grounds of effects described with a few other phthalates.

***In vitro* study**
DEP failed to produce any effect on testicular Sertoli cell function or on testicular cell cultures contrary to other phthalate esters tested. The slight effects noted were not conclusive because the high doses used and the insufficiency of investigation.
In human Spermatozoae, transferred into a defined medium, DEP can reduced moderately and transiently the motility of the cells, but in a less extent than other phthalates. No toxicity was observed.
No effect was demonstrated on hepatic cytochrome P-450 content of young male rats treated by DEP.

***In vivo* studies**
Testosterone levels in serum and testes were decreased in rats fed 2% DEP (approximately 1000 mg/kg/day) in the diet for a week, but no testicular damage occurred as evidenced by testes weight or testes zinc content.
Rats receiving 2000 mg/kg/day DEP by oral gavage for two days showed no effect on seminiferous tubular structure or Leydig cell morphology by light microscopy. Ultrastructural examination of Leydig cells showed mitochondrial swelling and focal dilatation of smooth endoplasmic reticulum. LH-stimulated testosterone secretion from Leydig cells incubated with the monoester of DEP was not affected.
Comment: a variety of potential effects of DEP have been reported otherwise, but the significance of the effects noted using in vitro techniques is questionable in view of the very high doses used, and the lack of effects after in vivo tests performed also at high doses.

Ref.: 27, 28, 30, 31, 32, 33, 39, 46, 72

2.6.2. Two-generation reproduction toxicity

No data have been reported.

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Toxicokinetics and Metabolism

Percutaneous penetration study in human skin *in vitro*
Percutaneous penetration of DEP was studied in human skin in vitro using the SAM system. The amount absorbed at 72h (% dose) was reported to be 4.7% in unoccluded and 3.5% in occluded skin. Percutaneous penetration in rat skin was significantly higher (37.5%) than in human skin. In epidermal sheets, the permeability constant of DEP was 1.14×10^{-5} cm/h for human skin and 37.00×10^{-5} cm/h for rat skin. The steady state absorption rate was 1.27 $\mu g/cm^2/h$ for human skin and 41.37 $\mu g/cm^2/h$ for rat skin. Thus DEP could facilitate the passage the skin of some other ingredients.

Rabbit
Application of ^{14}C-DEP on the shaved backs of rabbits resulted in about 27% excretion in urine in

the first 24 hours and a cumulative excretion in urine of about 49% and in faeces of about 1% over 4 days. Blood levels accounted for about 7% of the dose after 1 hour of application and less than 1% of the dose after 4 days. Tissue distribution showed the greatest accumulation in kidney and liver.

Oral administration

Rat and Mouse

Oral administration of ^{14}C-DEP to rats and mice resulted in maximum concentrations of radioactivity in kidney and liver, followed by blood, spleen and fat. Highest levels were observed within 20 minutes, followed by fairly rapid decrease to only trace amounts at 24 hours. Excretion occurred primarily in urine. Cumulative urinary and faecal excretion, respectively, was 47 and 0.7% within 12 hours, 82 and 2.5% within 24 hours and 90 and 2.7% within 48 hours after the dose. Absorbed DEP is distributed throughout body tissues with the greatest accumulation of the dose in the kidney and liver. Major metabolism is by partial hydrolysis to ethanol and the mono-ester mono-ethylphthalate, which is fairly rapidly excreted in the urine.

Metabolism after oral administration of DEP to rats results in hydrolysis with the principal urinary metabolite being mono-ethyl phthalate and with phthalic acid as the minor secondary urinary metabolite. Hydrolysis to the mono-ester can occur in the lumen of the gastrointestinal tract or in intestinal mucosal cells after oral administration as well as in organs such as the liver, kidney and lung after systemic absorption. Hydrolysis to the mono-ester by skin has been demonstrated using *in vitro* percutaneous absorption though rat skin and adult human skin.

The specific enzymes involved in the hydrolysis of DEP to the monoester are not well characterized for various species:

Human plasma-derived arylesterase did not hydrolyse DEP. DEP was hydrolyzed to its monoester by purified carboxylesterase from human liver and rat liver. Microsomal carboxylesterase activity towards DEP was induced in mouse liver and rat kidney but not in rat or mouse testes. In clofibrate-fed animals authors isolated a novel esterase from mouse hepatic microsomes having high catalytic activity compared with the mouse hepatic microsomes. DEP was hydrolysed to the mono-ester, but the mono-ester was not hydrolysed even after prolonged incubation periods.

Limited evidence for induction of enzymes by DEP has been reported. Preincubation of DEP in microsomal pellets and supernatant isolated from Sprague-Dawley males treated with phenobarbital intraperitoneally for 3 days, had no effect on cytochrome P450 or on N-acetyl transferase activity in rat liver microsomal suspensions, but the activity of UDP glucuronyl transferase was reduced. Increased activity of peroxisomal enzyme carnitine acetyl transferase was observed in rat primary hepatocyte cultures in the presence of DEP. Male rats fed 2% DEP in their diet for 3 weeks showed marginal hepatic peroxisome proliferation.This was confirmed by only a slight decrease in serum lipids (cholesterol and triglycerides). It is well established, only those compounds producing hepatic peroxisome proliferation are decreasing both serum lipids.

Ref.: 5, 12, 29, 32, 41, 42, 44, 48, 49, 50, 53, 54, 60, 62, 63, 64, 65, 68, 69, 83, 101, 103

2.8. Mutagenicity/Genotoxicity

***Salmonella typhimurium* bacterial gene mutation assay**

Strains:	TA100, TA1535, TA1537, TA98	
Metabolic activation:	-S9; + 10 % hamster S9; + 10 % rat S9	
Replication:	2 experiments; 2 laboratories (4 replicates)	
Doses:	0, 10, 33, 333, 667, 1000 and 3333 µg/plate	2 exp.
	0, 100, 333, 1000, 3333 and 10000 µg/plate	2 exp.

Results
Negative results on all 4 strains and on all metabolic conditions, with some toxicity at the higher dose in 2 exp. Positive results have been obtained with appropriate controls.

Sister chromatid exchanges on Chinese hamster ovary cells grown in vitro.

	(- S9)	(+ S9)	(+ S9)
Dose:	5-17-50 µg/ml;	50-167-500 µg/ml;	167-500-750 µg/ml

Results
Positive in the presence of S9 ($P<0.001$) in both experiments

Chromosome aberrations in Chinese hamster ovary cells grown in vitro

Dose: 70-151-324 µg/ml ± S9-mix
(15.5 hours – S9; 17.5 hours + S9)

Results
negative

Conclusion
Diethylphthalate is clearly non mutagenic on Salmonella bacterial gene-mutation assay; it is positive for the induction of Sister chromatid exchanges in Chinese hamster cells. It is equivocal for the induction of chromosome aberrations on Chinese hamster cells grown in vitro, because only one experiment has been performed and has produced equivocal results in the absence of metabolic activation, at a very short harvest time.

Ref: 70, 112

2.9. Chronic Toxicity and Carcinogenicity

There is no unequivocal evidence for serious toxicity or carcinogenicity in rats or mice after long-term administration of DEP by the oral or dermal route of exposure.

2.9.1. Dermal route of exposure

Rats
Carcinogenic effects of DEP were evaluated in a 2-year dermal study in male and female F344/N rats.

Rats were treated (60 males and 60 females per group) with undiluted DEP at doses of 0, 100 and 300 µl/rat/day (approximately 0, 112, and 336 mg/rat/day) applied dermally to clipped inter-scapular skin, five days per week for 103 weeks. An interim evaluation with sacrifice of 10 animals of each sex was done at 15 months.
Survival rate at 15 months was similar to that of controls. However, at 2-year it was significantly reduced in all male groups including controls.
No treatment related clinical signs were noted. However male, and in a less extent, female rats in all groups including controls showed weight loss, loss of appetite, hypo-activity, emaciation, requiring moribund sacrifice. Thus the relation to treatment is doubtful.
No evidence of dermal toxicity was noted.
The mean bodyweights of 300 µl treated males were slightly less than the controls throughout the study.

Incidences of Skin lesions of Rats in the 2-year Dermal Study

Dose (µl)	0	100	300
15-month Interim Evaluation			
Male			
Skin, site of application(a)	10	5	9
Acanthosis(b)	0	5** (1)(d)	6** (1)(d)
Female			
Skin, site of application and acanthosis	not examined	not examined	not examined
2-year study			
Male			
Skin, site of application	50	50	51
Acanthosis(b)	2(1.5)	5(1.4)	21**(1.1)
Female			
Skin, site of application	50	49	50
Acanthosis(b)	8(1.4)	1.8*(1.1)	23** (1.1)

* significantly different ($p \leq 0.05$) from the control group by the Fischer exact test (interim evaluation) or the log. regression analysis (2-year study)
** $p \leq 0.01$
a number of animals with skin examined microscopically
b number of animals with lesion d average severity grade of lesion in affected animals. 1 minimal, 2 mild, 3 moderate

As shown in the table above, a treatment-related increased incidence of minimal to mild epidermal acanthosis was observed at the site of application in both males and females at 15 months and 2-year evaluation periods. This lesion is probably an adaptative response to local irritation. In a few animals, minimal hyperkeratosis was associated with the acanthotic lesions.
The incidence of fatty degeneration of the liver was decreased in both male and female treated rats:

26/50, 8/50, 4/51 in males respectively for controls, 100 µl and 300 µl groups.

These decreased incidences may be attributed to the hypolipidemic action of DEP.

Decreased incidence of mammary gland fibro-adenomas occurred in female treated rats, and of interstitial cell tumours in treated males.
Skin neoplasms were not observed in female rats and were only rarely observed in male rats:

Kerato-acanthoma:	1/49 (controls), 1/50 (100 µl), 0/51 (300 µl)
Face papilloma:	1/51 (300 µl)
Lip papilloma:	1/51 (300 µl)
Thoracic, kerato-acanthoma:	1/51 (300 µl).

Conclusion
- for carcinogenic potential, the NOAEL is equal or higher than 300 µl per a rat (corresponding in mean to 1000 mg/kg for a rat of 350 g);
- for chronic effects, the NOAEL is equal to 100 µl per rat (corresponding in mean to 350 mg/kg for a rat of 350 g).

Mice were treated dermally with DEP doses of 0, 7.5, 15 or 30 µl (approximately 0, 9, 18 and 37 mg/ per animal). For each dose DEP was diluted in 100 µl acetone. The treatment was done five days /week for up to 103 weeks with a week recovery.
No significant evidence of toxicity or neoplasia was seen at the site of application.
An interim evaluation with sacrifice of 10 animals of each sex was done at 15 months.
The survival rate presented no difference in males between treated and control groups. In females a slight decrease of the survival rate was noted according to the dose (41/50 (controls), 38/51 (low dose), 37/49 (mid-dose), 36/49 (high dose). The bodyweight was not impaired by the treatment.

Incidences of Neoplasms and Nonneoplastic Lesions of the Liver of male Mice in the 2-year Dermal Study				
Dose µl	**0**	**7.5**	**15**	**30**
15-Month Interim Evaluation				
Liver(a)	10	3	1	10
Hepatocellular adenoma(b)	1	2	1	2
Hepatocellular carcinoma	0	0	0	1
2-year Study				
Liver	50	50	50	50
Basophilic focus	0	1	9**	3
Hepatocellular adenoma				
Overall rate (d)	6/50(12%)	11/50(22%)	9/50(18%)	12/50(24%)
Terminal rate(e)	6/43(14%)	10/41(24%)	9/46(20%)	12/43(28%)
Log.regression analysis	p=0.140	p=0.118	p=0.337	p=0.094
Hepatocellular carcinoma				
Overall rate (d)	4/50(8%)	4/50(8%)	6/50(12%)	7/50(14%)
Terminal rate (e)	3/43(7%)	1/41(2%)	5/46(11%)	3.43(7%)
Log.regression analysis	p=0.170	p=0.623	p=0.369	p=0.257
Hepatocellular adenoma and carcinoma(g)				
Overall rate (d)	9/50(18%)	14/50(28%)	14/50(28%)	18/50(36%)
Terminal rate (e)	8/43(19%)	11/41(27%)	13/46(28%)	14/43(33%)
Log.regression analysis	p= 0.040	p=0.144	p=0.206	p=0.034

** significantly different ($p \leq 0.01$) from the control group by logistic regression analysis.
a number of animals with liver examined microscopically
b number of animals with lesions
d number of animals with neoplasms per number of animals with liver examined microscopically
e Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for incurrent mortality
g Historical incidence for 2-year study with untreated control groups.

Incidences of Neoplasms and Nonneoplastic Lesions of the Liver of Female Mice in the 2-year Study				
Dose (µl)	**0**	**7.5**	**15**	**30**
15-Month Interim Sacrifice				
Liver	10	4	3	10
Hepatocellular adenoma	3	0	0	1
Hepatocellular carcinoma	0	0	0	1
2-Year Study				
Liver	50	51	50	50
Basophilic focus	2	3	6	2
Hepatocellular adenoma				
Overall rate	4/50(8%)	12/51(24%)	14/50(28%)	10/50(20%)
Terminal rate	4/41(10%)	11/38(29%)	12/37(32%)	7/36(19%)
Logistic regression analysis	p=0.127	p=0.017	p=0.006	p=0.075
Hepatocellula r carcinoma				
Overall rate	4/50(8%)	5/51(10%)	6/50(12%)	3/50(6%)
Terminal rate	2/41(5%)	2.38(5%)	2.37(5%)	0/36(0%)
Logistic regression analysis	p=0.297	p=0.603	p=0.457	p=0.484
Hepatocellular adenoma or carcinom a(h)				
Overall rate	7/50(14%)	16/51(31%)	19/50(38%)	12/50(24%)
Terminal rate	5/41(12%)	12/38(32%)	14/37(38%)	7/36(19%)
Logistic regression analysis	p=0.231	p=0.029	p=0.005	p=0.161

h historical incidence

In summary an increased incidence of basophilic foci in the liver was noted in mid-dose male mice with no dose-related trend, but not in females.
As evidenced above, a marginal increased incidence of combined hepatocellular adenoma or carcinoma was observed in high-dose male mice. In females, the incidence of combined hepatocellular adenoma or carcinoma was higher in low and mid-dose mice than in high-dose mice or controls.
Because the incidence of hepatocellular neoplasms in the high-dose male mice was similar to the historical control mean, and because there was no dose response for liver neoplasms in female mice, these marginal increases were considered to be uncertain findings providing only equivocal evidence of carcinogenic activity. No other lesions or neoplasms showed a relation to treatment.

Conclusion
- for carcinogenic potential, the NOAEL is equal or higher than 30 µg/l per a mouse (corresponding in mean to 1057 mg/kg for a mouse of 35 g);
- for chronic effects, the NOAEL is equal to 15 µl per male mouse and 30 µl per female mouse. These numbers correspond approximately to 514 mg/kg (males) and 1057 mg/kg (females) for a mouse of 35 g).

A one-year initiation-promotion study in a group of 50 male mice was conducted to evaluate the potential of DEP applied dermally to initiate tumorigenesis when followed by a strong promoter (TPA: 12-*O*-tetradecanoylphorbol-13-acetate) or to promote tumorigenesis following administration of a known initiator (DMBA: 7,12-dimethylbenz (a)anthracene). Initiators were applied once during the first week. Promoters were generally applied 3 to 5 times a week, from week 2 to the end of the study. All doses were applied at a volume of 0.1 ml. Apart from the treated groups, the study included the following groups: vehicle control (acetone/acetone),

initiation/promotion control (DMBA/TPA), initiator control (DMBA/acetone), and promoter control (acetone/TPA).

Incidences given in the table below are for lesions which occurred at the site of application.

Incidences of Skin Lesions and Neoplasms of Male Mice in the 1-year Initiation/Promotion Dermal Study							
	Acanthosis	**Ulceration**	**Exudate**	**Hyperkeratosis**	**Squamous Cell Papilloma**	**Squamous Cell Carcinoma**	**Squamous Cell Papilloma or Carcinoma**
Vehicle control acetone/acetone	8 out of 50	2 out of 50	4 out of 50	1 out of 50	0 out of 50	0 out of 50	0 out of 50
Initiation controls							
Acetone/DEP	9 out of 50	5 out of 50	8 out of 50	6 out of 50	0 out of 50	0 out of 50	0 out of 50
Promotion controls							
DEP/acetone	14 out of 49	6 out of 49	11 out of 49	8 out of 49*	1 out of 50	0 out of 50	1 out of 50
DEP Initiation							
Acetone/TPA	47 out of	23 out of	25 out of	34 out of	5 out of 50*	0 out of 50	5 out of 50*
DE P/TPA	43 out of	25 out of	32 out of	31 out of	3 out of 49*	0 out of 49	3 out of 49*
DEP Promotion							
DM BA/acetone	18 out of	7 out of 50	10 out of 50	13 out of	1 out of 50	2 out of 50	3 out of 50
DM BA/DEP	6 out of 50	5 out of 50	5 out of 50	5 out of 50	2 out of 50	0 out of 50	2 out of 50
Initiation/Promotion Control							
DM BA/TPA	46 out of 49*	22 out of 49*	32 out of 49*	40 out of 49*	23 out of 49* a, b	7 out of 49* a, b	25 out of 49* a, b

* significantly different ($p \leq 0.05$) from the vehicle control group (acetone/acetone) by log. regression
a significantly different ($p \leq 0.05$) from the promotion control group (DMBA/acetone) by log. regression
b significantly different ($p \leq 0.05$) from the initiation control group (acetone/TPA) by log. regression

Based on the incidence of skin neoplasms diagnosed histologically and the multiplicity of skin neoplasms, there was no suggestion that DEP was able to initiate skin carcinogenesis when chronically promoted by TPA.
Further there was no evidence that DEP was able to promote skin carcinogenesis in skin previously initiated by DMBA.
Thus, no initiating or promoting activity of DEP was demonstrated.

2.9.2. Oral route of exposure

Rats (albino rats from Food Research Lab.) were given 0.5, 2.5 or 5% DEP (approximately 250, 1250 or 2500 mg/kg/day) in diet for two years.
At two-year period 42% of the animals of each group were still alive.
At the highest dose the rats showed slightly decreased body weight gain throughout the study and diminished efficiency of food utilization compared with the control rats. No treatment-related: effects on hemo-cytology, blood sugar, non-protein nitrogen-levels or urinalyses were observed. Postmortem examinations of dead or sacrificed rats revealed no unusual pathology, either gross or microscopic, which appeared to bear any relation, to the DEP in the diet, but more related to infectious diseases. Comment: the study suffered from a few deficiencies and has not been well reported

NOAEL for chronic effect = 1250 mg/kg for both males and females
NOAEL for carcinogenicity = ≥ 2500 mg/kg

Ref.: 59, 70, 77

2.10. Estrogenic potential

- DEP has not been reported to cause estrogenic activity in vertebrates, although weak activity has been reported in some *in vitro* studies.
- EPA (1996) determined that there was insufficient evidence, at that time, to demonstrate that DEP causes hormonal disruption.

Groups of ten immature (21-22 days old) female Wistar [Crl (WI) BR] rats received a single oral dose of 0 (vehicle control), 50, 150 or 500 mg/kg body weight of DEP once a day for 3 consecutive days. As a positive control, one group of rats received a single oral dose of 0.4 mg β-estradiol/kg body weight once a day for 3 consecutive days. The vehicle used for DEP and β-estradiol was peanut oil. Approximately 24 hours after administration of the final dose the rats were sacrificed, the uterus removed and weighed. There were no treatment-related effects of DEP on clinical observations or on body weights throughout the study. The uterus weights were unaffected by treatment with DEP, while the positive control produced a significant effect on uterus weight.

- Using an *in vitro* recombinant/receptor gene bioassay with HeLa cells stably transfected with the Gal 4-human oestrogen receptor chimeric construct, Gal 4 -HEGO and the Gal 4 -regulated reporter gene, 17m5-G-Luc, no significant induction in luciferase activity was observed with DEP.

- Using a recombinant yeast strain *(Saccharomyces cerevisiae)* containing hER (the human oestrogen receptor) and the reporter gene, *lac-Z,* DEP did not demonstrate estrogenic potential over the range of concentrations (10^{-8} - 10^{-4} molar) tested. The results were compared against the positive control, β-estradiol, and the negative control, testosterone.

- An *in vitro* oestrogen receptor-binding assay using rat uterine cytosol from the uteri of ten-week old Wistar rats was conducted. The assay measures the potential binding of DEP to the oestrogen receptor by testing its ability to compete with and displace ^{3}H-17β-estradiol bound to the receptors in the cytosol. The results indicated that DEP did not bind to the oestrogen receptor.

- No estrogenic activity using the oestrogen-responsive human breast cancer cell line ZR-75 was reported. A slight increase in cell proliferation at day 8 was observed, but not at days 5 or 12 using the concentration of 10^{-5} molar DEP with the oestrogen-responsive human breast cancer cell line MCF-7. They also reported an extremely weak estrogenic activity using yeast cells with the human oestrogen receptor. Activity was observed only at concentrations greater than 10^{-4} molar (a potency only 0.0000005 that of 17 β-estradiol).

- Some oestrogen-mimicking xenobiotics in vertebrates can also affect the hormonally regulated molting process in arthropods by binding and blocking the steroid receptors. DEP was reported to delay the molting in the water flea, *Daphnia magna.* at a concentration of 22.4 mg/l (over 100 times the concentration causing inhibition by diethylstilbestrol). It was also reported that DEP at 50 mg/l inhibited the chitobiase activity involved in the premolt stage of the fiddler crab, *Uca pugilator.*

Ref.: 6, 37, 100, 113, 114.

2.11. Safety evaluation

CALCULATION OF THE MARGIN OF SAFETY

(Diethylphthalate)

(Cosmetic products/ ethanol denaturant/ fragrance solvent)

Because of the wide spread use of Diethyl phthalate, the SCCNFP made a worst case calculation.
Based on a usage volume of 10 ml, containing at maximum 10%:

Maximum amount of ingredient applied	**I (mg)**	**=**	**1120 mg**
Typical body weight of human		**=**	**60 kg**
Maximum absorption through the skin (Human skin in vitro)	**A (%)**	**=**	**5%**
Dermal absorption per treatment	**I x A**	**=**	**56 mg/day**
Systemic exposure dose (SED)	**I x A/60 kg**	**=**	**0.93 mg**
No observed adverse effect level (rat, oral, 16 week)	**NOAEL**	**=**	**150 mg /kg bw**
Margin of Safety	**NOAEL / SED**	**=**	**161**

These data have to be compared to the results published in 2001 (Ref.115), referring to a paper mentioning a survey on more than 2000 perfumes compounds intended for hydro-alcoholic cosmetics, and reported a 97.5 percentile of use for DEP of 28.6%. The author concluded to a potential exposure of approximately of 44 mg/day corresponding to 0.73 mg/kg/day, giving a MOS of 205.

Other examples
Ethanol denaturant
DEP can also be used as ethanol denaturant at a maximum concentration of 1% (hypothetic usage volume of 10 ml), from which it results a 5.6 mg/d potential exposure giving a **MOS of 1607.**

Fragrance solvent
DEP can also be used as fragrance solvent at a maximum concentration of 50% (hypothetic usage volume of 1 ml), from which it results a 28 mg/d potential exposure giving a **MOS of 321.**

2.12. Conclusions

DEP shows a low level of toxicity. Testing for dermal irritation and sensitisation in humans as well as in animals, and for photo-toxicity and photo sensitisation in human volunteers, has demonstrated its safety of use. Even undiluted the effects observed were minimal or moderate.

The results of sub-chronic, and reproduction studies did not show any adverse effects attributable to treatment. The marginal increase of combined hepatocellular adenomas and carcinomas in high-dose male mice was considered (Ref.70) as an uncertain finding due to the absence of effect

on females and the low incidence observed in controls. In rats no effect was noted on that end-point. Though all the genotoxicity end-points were not fully covered, the weight of evidence supports a low level of concern in carcinogenicity of DEP under the normal conditions of use, based also on borderline effects observed in some genotoxicity tests.

2.13. Opinion

The SCCNFP is of the opinion that the safety profile of Diethyl-phthalate supports its use in cosmetic products at current levels.

At present the SCCNFP does not recommend any specific warnings or restrictions under the currently proposed conditions of use.

2.14. References

Global references

Are put in italic characters publications or reports discarded for various reasons : poor scientific quality, bad reporting, document too much summarised, original language other than English with no translation, or several insufficiencies which do not allow to draw any valuable scientific conclusions.
References 115, 116 and 117 have been placed at the end of the list, because they have been brought to our attention recently.

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