Literature Search Product
For
Diethyl Phthalate (DEP)

(CAS No. 84-66-2)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

Project 06-26

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Search Strategy and Presentation of Results

The Statement of Work provided by EPA called for a literature search product for diethyl phthalate (DEP). A literature search was conducted in July 2007. PubMed, Toxline, Biological Sciences Database, and Science Direct Database were searched, resulting in 182 unique hits after any duplicate records were removed. No limitation to publication year was applied. The following search terms were used: diethyl phthalate, 84-66-2, and toxicity, toxic*, toxico*, cancer*, carcinog* , carcinoma* , oncogene*, tumor*, neoplasm*, mutag*, mutat*, genotox*, fetotox*, embryotox*, teratol*, teratogen*, reproduct*, developmental, pK, multigeneration, neurotox*, immunotox*, pharmacokinetic*, pharmacodynamic* , PBPK, metabolism, epidemiol*, human, systemic, dermal, and intravenous. An additional 15 studies of interest were identified from the ATSDR *Toxicological Profile for Diethyl Phthalate* (1995) and other recent review documents and added to the reference database. Of these results, 115 were considered potentially of interest to the development of the Toxicological Review.

The final number of 72 relevant references is presented in the Literature Search Product (clearly off-topic studies, such as environmental media levels and ecological effects, were not included here) and all references identified by the literature searches are presented in the accompanying RefMan file. The following Literature Search Product presents all of the identified references categorized as suggested in the 2006 IRIS Toxicological Review template. Some references are relevant to multiple sections, which is indicated parenthetically in bold following the abstract (e.g., “also 4.6”). In Section 3, a number of references are placed in an initial general section on kinetics because they refer to more than one of the categories absorption, distribution, metabolism, elimination, or PBPK modeling.

Yellow highlights were added to the references that we consider central to the development of this IRIS Toxicological Review. References not highlighted represent various categories: those that contain the chemical name or CASRN without evident relevance to the project as judged from title and/or abstract; abstracts to which a full peer-reviewed publication exists; and references that may be used as needed to extract additional information.

As an introduction to this Literature Search Product, there are a few items of particular note to report. Since the development of the IRIS summary (last revised in 1993) and ATSDR’s Toxicological Profile (1995), a few studies have been recently published examining the potential reproductive and developmental effects of diethyl phthalate (including 2 two-generation studies and 1 three-generation study) which may help to fill an existing data gap for this chemical. In addition, a review article was recently published (Api, 2001) that largely focused on potential effects resulting from dermal exposure due to its common use as a vehicle for fragrance and cosmetic ingredients; numerous unpublished industry studies were included among its citations. No data on inhalation effects, PBPK models, or susceptible populations were identified.

Secondary Material (review articles or overviews from other agencies) applying to several or all of Sections 2-4.

Anonymous. (1985) **Final report on the safety assessment of dibutyl phthalate, dimethyl phthalate, and diethyl phthalate.** J Am Coll Toxicol 4 Dibutyl Phthalate (DBP), Dimethyl Phthalate (DMP), and Diethyl Phthalate (DEP) are dialkyl phthalates used primarily in cosmetics at concentrations of less than 10 percent as plasticizers, solvents, and perfume fixatives. The mutagenic activity of DBP, DMP, and DEP toward Salmonella typhimurium mutants is essentially negative, but some assays reported positive findings. Carcinogenesis was not observed in DPB feeding studies. Limited clinical data on DBP, DMP, and DEP indicate that these ingredients are not human skin irritants, sensitizers, or phototoxic agents. On the basis of the available data, these compounds are safe for topical application in the present practices of use and concentration in cosmetics.

Api, AM. (2001) **Toxicological profile of diethyl phthalate: a vehicle for fragrance and cosmetic ingredients.** Food Chem Toxicol 39:97-108. Diethyl phthalate (DEP; CAS No. 84-66-2) has many industrial uses, as a solvent and vehicle for fragrance and cosmetic ingredients and subsequent skin contact. This review focuses on its safety in use as a solvent and vehicle for fragrance and cosmetic ingredients. Available data are reviewed for acute toxicity, eye irritation, dermal irritation, dermal sensitization, phototoxicity, photoallergenicity, percutaneous absorption, kinetics, metabolism, subchronic toxicity, teratogenicity, reproductive toxicity, estrogenic potential, genetic toxicity, chronic toxicity, carcinogenicity, in vitro toxicity, ecotoxicity, environmental fate and potential human exposure. No toxicological endpoints of concern have been identified. Comparison of estimated exposure (0.73 mg/kg/day) from dermal applications of fragrances and cosmetic products with other accepted industrial (5 mg/m(3) in air) and consumer exposures (350 mg/l in water; 0.75 mg/kg/day oral exposure) indicates no significant toxic liability for the use of DEP in fragrances and cosmetic products.

ATSDR. (1995) **Toxicological profile for diethyl phthalate.**

BIBRA working group. (1994) **Diethyl phthalate.** In humans, diethyl phthalate was irritant to the eyes and to broken, but not intact, skin. One individual became sensitized following repeated contact with articles containing diethyl phthalate although attempts to induce skin sensitization in volunteers were unsuccessful. In the liquid form, the phthalate was mildly irritant to guinea-pig skin and rabbit eyes, and irritation to the respiratory passages and eyes of cats was seen following exposure to airborne diethyl phthalate. A low acute toxicity was reported in rats exposed orally. Central nervous system effects and damage to the spleen and kidneys occurred in laboratory animals given single injections of diethyl phthalate and the liver was affected in subjects exposed through dialysis equipment. Repeated feeding to rats caused blood effects and produced increases in several organ weights (including the testes and liver), liver peroxisomes (subcellular structures), and liver enzyme activities. Effects seen in rats and mice after repeated dermal exposures included increases in liver and kidney weights and changes in the liver tissue of mice. In cats inhaling the phthalate repeatedly, there were central nervous system effects and cellular changes in the blood, liver and kidneys. In a multi-generation study, decreased sperm count and reduced litter size occurred in subsequent generations of mice fed diethyl phthalate. Repeated dietary administration to pregnant rats affected foetal development at maternally toxic doses; foetal development was also affected when pregnant rats were treated by repeated intraperitoneal injections. No evidence of carcinogenic activity has been found in rats exposed either orally (in limited studies) or dermally, although benign liver tumours have been seen in mice after repeated dermal administration. Diethyl phthalate caused chromosome damage to mammalian cells in culture but only in the presence of a metabolizing system. A number of investigators have reported evidence of weak mutagenicity in Ames bacterial tests.

CEC. (1993) **Diethyl phthalate.** The toxicology of chemicals. 2. Reproductive toxicity Vol:1. In animals, no reproductive or embryofetotoxic effects of diethyl phthalate exposure were observed at non-toxic doses. There are no relevant data available to assess the reproductive toxicology of diethyl phthalate to humans.

EASTMAN KODAK CO. (8/78) **TOXICITY AND HEALTH HAZARD SUMMARY.** 878214401 OTS0206525 ACUTE TOXICITY, RABBITS, ORAL; ACUTE TOXICITY, MICE, ORAL; ACUTE TOXICITY, MICE, INTRAPERITONEAL; PRIMARY DERMAL IRRITATION, GUINEA PIGS, DERMAL; PRIMARY DERMAL

SENSITIZATION, GUINEA PIGS, DERMAL; ACUTE TOXICITY, GUINEA PIGS, DERMAL; ACUTE TOXICITY, RATS, INHALATION

EPA. (1980) **Ambient water quality criteria for phthalate esters.** EPA 440/5-80-067 Acute freshwater test results were available for five phthalate esters, and these were conducted with a relatively small diverse group of freshwater fish and invertebrate species. The acute values, with one exception, all exceeded 1,000 ug/l. Sensitivity differences were generally similar for the tested freshwater species. No final acute values are calculable for any ester since the minimum data base requirements were not met. Chronic freshwater test results were available for two phthalate esters. The chronic values for butylbenzyl phthalate were 220 and 440 ug/l with the calculated acute-chronic ratios being 17 and 42. The chronic values for di- 2-ethylhexyl phthalate were 3 and 8.4 ug/l and no acute-chronic ratios were calculable. No final chronic values could be determined. Plant test results were available for three phthalate esters. The plant values for diethyl and dimethyl phthalate were similar to the acute results for these phthalates and invertebrate species. A wide variation was found in the EC50 values for butylbenzyl phthalate, which values ranged from 110 to 1,000,000 ug/l. Residue test results were available for five phthalate esters. A wide variation was found for bioconcentration values for both the invertebrate (14-2,680) and fish (42-886) species. More residue data were available for di-2-ethylhexyl phthalate than for the other ester. Additional freshwater toxicity results were available with four phthalate esters. None of these data showed toxicity values lower than those already discussed. Acute saltwater test results were available for three phthalate esters with one invertebrate and one fish species. The lowest concentrations at which acute effects were observed were 900 ug/l for butylbenzyl phthalate and 7,590 ug/l for diethyl phthalate, both for mysid shrimp, and 58,000 ug/l for dimethyl phthalate with the sheepshead minnow. There were no saltwater chronic or residue test results for any phthalate ester. Effects of phthalate esters on saltwater algal species were reported at concentrations as low as 3.4 ug/l. Aquatic Life: The available data for phthalate esters indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 940 and 3 ug/l, respectively, and would occur at lower concentrations among species that are more sensitive than those tested. The available data for phthalate esters indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 2,499 ug/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of phthalate esters to sensitive saltwater aquatic life but toxicity to one species of algae occurs at concentrations as low as 3.4 ug/l. Human Health: For the protection of human health from the toxic properties of dimethyl phthalate ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 313 mg/l. For the protection of human health from the toxic properties of dimethyl phthalate ingested through contaminated aquatic organisms alone, the ambient water criterion is determines to be 2.9 g/l. For the protection of human health from the toxic properties of dimethyl phthalate ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 350 mg/l. For the protection of human health from the toxic properties of dimethyl phthalate ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 1.8 g/l. For the protection of human health from the toxic properties of dibutyl phthalate ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 34 mg/l. For the protection of human health from the toxic properties of dibutyl phthalate ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 164 mg/l. For the protection of human health from the toxic properties of di-2-ethylhexyl phthalate ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 15 mg/l. For the protection of human health from the toxic properties of di-2-ethylhexyl phthalate ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 50 mg/l.

EPA. (1987) **Health effects assessment for selected phthalic acid esters.** EPA/600/8-88/053 Selected physical and chemical properties and available half-life data or dimethyl, diethyl, di- n-butyl, di-n-octyl, bis(2-ethylhexyl) and butyl enzyl phthalate are presented in Table 1-1. The chemicals can be classified as benzenedicarboxylic acid diesters (U.S. EPA, 1980b-g). In air, gaseous phthalic acid esters are predicted to react with hydroxyl radicals. The actual atmospheric half-life because of adsorption into airborne particulate matter may be longer than the estimated half-life given in Table 1-1. Removal of phthalate esters from the atmosphere by wet and dry deposition has been observed (Kawamura and Kaplan, 1983; Altas 1981; Karasek et al., 1978; Weschler, 1984). In water, the phthalate esters apparently biodegrade, volatilize, adsorb to sediments and bioconcentrate depending upon the size and complexity of the ester chains, environmental conditions and presence of fulvic acids (Callahan et al., 1979). Studies indicate that phthalic acid exters undergo rapid primary degradation and mineralization (half-lives range from days to weeks) by bacteria commonly found in the environment (Saeger and Tucker, 1973a,b; Gledhill et al., 1980). The rates of degradation have been shown to decrease with increasing size and complexity of the phthalate chain.

Monitoring data and sediment-water partitioning coefficients indicate that adsorption to suspended solids and sediments is also likely to occur for all the phthalates (Sullivan et al., 1982; Maybe et al., 1981), although complexation with fulvic acid can cause solubilization (Khan, 1980; Ogner and Schnitzer, 1970; Matsuda and Schnitzer, 1971). Phthalic acid ester half-lives in soil were estimated from biodegradation data from a garden soil study (Shanker et al., 1985). Removal by mechanisms other than biodegradation, however, may also be significant from lower molecular weight phthalates. For example, dimethyl and diethyl phthalate are predicted to be highly mobile in soil and also have the potential to volatilize from dry soil surfaces. Overall, short-chain phthalates biodegrade at a faster rate than longer chain phthalates and anaerobic degradation is slower than aerobic degradation (Shanker et al., 1985). Applying an uncertainty factor of 10 for interspecies extrapolation and 10 for interindividual variability results in an RfDSO of 8 mg/kg/day or 525 mg/day. Di-n-butyl phthalate. U.S. EPA (1980a) derived an ADI of 0.1 mg/kg/day based on a 52-week oral rat NOAEL of 125 mg/kg/day (Smith, 1953) and an uncertainty factor of 1000. A higher dose (1.25%in the diet or 625 mg/kg/day) caused 50% mortality within 1 week of the initial exposure (Smith, 1953). Oral. Evidence of oncogenic potential sufficient for computation of carcinogenic potency has been generated only for bis(2-ethylhexyl) phthalate (NTP, 1982a). Using data from NTP (1982a), carcinogenic potenciescan be derived from combined hepatocellular carcinoma and neoplastic nodules in rats, and combined hepatocellular carcinoma and adenoma in mice using the computerized multistage model developed by Howe and Crump (1982). The highest value, an adjusted human interim carcinogenic potency of 8.36x10-3 (mg/kg/day)-1 was obtained from data on male mice (Table 6-7). Turnbull and Rodricks (1985) have cautioned that using rodent data to estimate bis(2- ethylhexyl) phthalate-promoted carcinogenic risk to humans may overestimate the actual risk. This caution was based on several factors including differences between rodents and primates in the metabolism of bis(2-ethylhexyl) phthalate, a nonlinear relationship between the administered dose of bis(2-ethylhexyl) phthalate and the dose of the hypothesized 'proximate carcinogenic species' in rodents, the fact that the hypothesized proximate carcinogenic species is produced to a greater extent in rodents than in primates, and differences in target site sensitivities between humans and rodents for liver tumors in general. These factors have not yet been evaluated by the EPA. As a result, the proposed interim carcinogenic potency value may be revised at a later date. Di(2-ethylhexyl)phthalate is a Group B2 carcinogen using the U.S. EPA weight-of-evidence approach, meaning that there are sufficient animal data and the compound is considered a probable human carcinogen. NTP (1982b) conducted cancer bioassays of butyl benzyl phthalate in both F344/N rats and B6C3Fl mice. No treatment related increases in incidence of either neoplastic or non-neoplastic lesions were noted in either sex of mice. In rats, increased mortality due to 'unexplained internal hemorrhage' was observed in treated males beginning at the 14th treatment week. This portion of the study was terminated after 28 weeks. Female rats showed increased incidence of mononuclear cell leukemia and leukemia or lymphoma at the high dose (1200 ppm). Since the background incidence of leukemia is normally high in F344/N rats, and because decreases in incidence of malignant lymphoma, all lymphoma or leukemia were seen in male mice this was considered to be equivocal evidence of carcinogenicity. Butyl benzyl phthalate is considered a Group C, possible human carcinogen using the U.S. EPA weight-of-evidence classification system. Data were considered inadequate for quantitative risk assessment. Inhalation. The oncogenicity of inhaled phthalate esters has not been tested. (Shortened)

Hauser, R; Calafat, AM. (2005) **Phthalates and human health.** Occupational and Environmental Medicine 62:806-818. Phthalates are a group of man-made chemicals with a wide spectrum of industrial applications. High molecular weight phthalates are primarily used as plasticizers in the manufacture of flexible vinyl and low molecular weight phthalates are used in personal-care products, as solvents and plasticizers for cellulose acetate, and in making lacquers, varnishes, and coatings. Animal and human studies indicate that potential human health effects include developmental anomalies, male and female reproductive health effects and respiratory health effects. Occupational studies on the health risks of phthalates are very limited.

International Programme on Chemical Safety (IPCS). (2003) **Diethyl phthalate.** Concise International Chemical Assessment Document (CICAD), Vol. 52. This CICAD on diethyl phthalate was developed primarily based on the evaluation available in the report Toxicological profile for diethylphthalate (ATSDR, 1995). Data identified up to the end of 1994 were covered in the review. A BUA (1994) report on diethyl phthalate was also available to the authors as reference , material. A further literature search was performed in October 2001 to identify any relevant information published after the original review. Information on the preparation and peer review of the source document is presented in Appendix 1. Information on the peer review of this CICAD is presented in Appendix 2. This CICAD was approved as an international assessment at a meeting of the Final Review Board, held in Ottawa, Canada, on 29 October - 1 November 2001. Participants at the Final Review Board meeting are listed in Appendix

3. The species sensitivity distribution method used to characterize the environmental risks is described in Appendix 4. The International Chemical Safety Card on diethyl phthalate (ICSC 0258), produced by the International Programme on Chemical Safety (IPCS, 2001), has also been reproduced in this document. Diethyl phthalate (CAS No. 84-66-2) is a colourless liquid with a slight aromatic odour and low volatility. It is soluble in water (1000 mg/litre at 25 degrees C). Diethyl phthalate is used as a plasticizer in a wide variety of consumer products, including plastic packaging films, cosmetic formulations, and toiletries, as well as in medical treatment tubing. As a result of its use, human exposure 'to diethyl phthalate is expected to be significant. Diethyl phthalate is likely to undergo biodegradation in the environment. Compared with other phthalates, it has a much lower capacity for binding to aquatic sediments, with between 70% and 90% of diethyl phthalate estimated to be found in the water column. Diethyl phthalate was detected in surface water at concentrations ranging from less than 1 to 10 ug/litre and in drinking-water at concentrations ranging from 0.01 to 1.0 ug/litre. Fish collected from the Great Lakes area in the USA contained diethyl phthalate at concentrations up to 1.7 mg/kg. Diethyl phthalate is not likely to biomagnify through the fond- chain. In a recent duplicate-portion study in Japan, the average intake of diethyl phthalate in hospital diet was estimated to be 0.35 pg/day per person, which probably was a result of contact between plastic packaging or gloves and the fond. General population exposure in the USA, as estimated from urinary concentrations of the monoester, was estimated to be 12 ug/kg body weight per day (median value). Leaching of diethyl phthalate from plastic tubing used in medical treatments reached 20 ug/litre with 1 h of perfusion with aqueous electrolyte solution, levels decreasing with extended perfusion time. Dermally applied diethyl phthalate penetrates the skin and can be widely distributed in the body, but it does not accumulate in tissue. Diethyl phthalate is hydrolysed in the body to the monoester derivative. Hydrolytic metabolism of diethyl phthalate is qualitatively similar in rodents and humans. LD50s for diethyl phthalate were 8600 mg/kg body weight and above following oral administration. Diethyl phthalate was a minimal to mild skin and eye irritant in experimental animals. Few cases of dermal irritation in humans after patch testing have been described; dermal sensitization has been described in humans, but seems to be rare. Slight increases in liver and kidney weights in rodents were observed following oral administration for up to 16 weeks. However, no adverse clinical chemical or histopathological changes were detected in the liver, kidney, or other organs in most studies. One 3-week study in rats showed an increase in liver weight at 1753 mg/kg body weight per day, which might be related to peroxisome proliferation. No carcinogenic effect was detected after dermal exposure in rats, and an equivocal response was observed in mice exposed dermally. No initiation or promotion activity of diethyl phthalate was detected in mice in a 1-year initiation/promotion study. Results of in vitro mutagenicity and clastogenicity studies were equivocal. No malformations but skeletal (rib) number variations were caused by an oral dose of 3215 mg/kg body weight per day in rats and a percutaneous dose of 5600 mg/kg body weight per day in mice - dose levels that also induced toxicity in the dams. No-observed-adverse-effect levels (NOAELs) of 1600 and 1900 mg/kg body weight per day were identified for mice and rats, respectively. A perinatal exposure to diethyl phthalate at 750 mg/kg body weight per day by gavage did not induce adverse effects in mothers or offspring and did not induce the malformations in male reproductive organs or the decreases in testis weights that were observed after exposure to other phthalates in the same study. In a continuous-breeding study, no adverse effects were detected in the F0 generation of mice following dietary administration of 3640 mg/kg body weight per day. However, decreased epididymal sperm concentration of the Ft generation and decreased number of live F2 pups per litter were caused by the administration of 3640 mg/kg body weight per day, together with mild inhibition of body weight gain and moderate increases in liver and prostate weights. Ultrastructural changes in the Leydig cells of rats were observed at an oral dose of 2000 mg/kg body weight per day administered for 2 days. No adverse immunological or neurological effects were reported in general toxicity studies. A tolerable intake of 5 mg/kg body weight was estimated from a NOAEL of 1600 mg/kg body weight per day for developmental effects to which an uncertainty factor of 300 was applied. The average daily intake of 0.35 ug/person (0.007 ug/kg body weight per day for a 50-kg person) derived in a hospital diet study in Japan is about 6 orders of magnitude lower than the tolerable intake. Exposure of the general population in the USA, estimated at 12 mg/kg body weight per day from monoethyl phthalate concentrations in urine, corresponds to 0.3% of the tolerable intake. The 95thpercentile value derived from the same study (110 ug/kg body weight per day) corresponds to 2% of the tolerable intake. Available data suggest that organisms in the freshwater aquatic environment are not likely to be at significant risk from exposure to diethyl phthalate, with measured concentrations in wastewater and surface water at least 1 order of magnitude lower than the pre-dicted no-effect concentration (PNEC) of 0.9 mg/litre. There are insufficient data available to estimate risk to marine organisms. Risk to soil organisms is also expected to be low, but data are inadequate to make a quantitative estimate.

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2. CHEMICAL AND PHYSICAL INFORMATION

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RTECS. (2007) **Registry of Toxic Effects of Chemical Substances..** National Library of Medicine, National Toxicology Information Program, Bethesda, MD. August 2007.

3. TOXICOKINETICS

Kawano, M. (1980) **Toxicological studies on phthalate esters. 2. Metabolism, accumulation and excretion of phthalate esters in rats.** Nippon Eiseigaku Zasshi 35:693-701.

Mose, T; Mortensen, GK; Hedegaard, M; et al. (2007) **Phthalate monoesters in perfusate from a dual placenta perfusion system, the placenta tissue and umbilical cord blood.** Reproductive Toxicology 23:83-91. Fetal exposure to phthalates may be associated with adverse reproductive effects, including cryptorchidism and decreased semen quality. Information about human placental transfer is needed to qualify the hypotheses. A dual recirculating placenta perfusion system to monitor concentrations of eight phthalate monoesters in fetal and maternal perfusates was established. In addition to perfusate background measures of phthalate monoesters, the concentrations in umbilical cord plasma and placenta tissue were measured. Monomethyl phthalate (mMP), monoethyl phthalate (mEP), monobutyl phthalate (mBP), and mono (2-ethyl- hexyl) phthalate (mEHP) were detected in both maternal and fetal perfusate, demonstrating a release of compounds from tissue or blood to perfusates. The distribution of compounds between perfusate, umbilical cord plasma, and tissue was in accordance with the physical-chemical properties of the compounds. Results from the present study of compounds residing in the tissue are essential before studying human transplacental transfer, storage, and metabolism of selected phthalate monoesters.

Singh, AR; Lawrence, WH; Autian, J. (1975) **Maternal-fetal transfer of 14C-di-2-ethylhexyl phthalate and 14C-diethyl phthalate in rats.** J Pharm Sci 64:1347-1350. 14C-Di-2-ethylhexyl and 14C-diethyl phthalates were administered intraperitoneally to pregnant rats on either Day 5 or 10 of gestation. Rats were sacrificed at 24-hr intervals starting on Days 8 and 11, respectively; maternal blood, fetal tissue, amniotic fluid, and placentas (whenever possible) were obtained. The 14C-activity of each sample was determined by scintillation counting. It was found that both diesters and/or their metabolic products were present in each of these compartments throughout the gestation period, thus suggesting that the embryo-fetal toxicity and teratogenesis reported previously could be the results of a direct effect of the compound (or its metabolites) upon developing embryonic tissue. Additionally, the reduction in concentration of 14C from these tissues as a function of time was found to fit a first-order excretion

curve. From this model curve, the half-life for both compounds was calculated; the average was about 2.33 days for di-2-ethylhexyl phthalate and 2.22 days for diethyl phthalate.

3.1. ABSORPTION

Elsisi, AE; Carter, DE; Sipes, IG. (1989) **Dermal absorption of phthalate diesters in rats.** Fundam Appl Toxicol 12:70-77. This study examined the extent of dermal absorption of a series of phthalate diesters in the rat. Those tested were dimethyl, diethyl, dibutyl, diisobutyl, dihexyl, di(2-ethylhexyl), diisodecyl, and benzyl butyl phthalate. Hair from a skin area (1.3 cm in diameter) on the back of male F344 rats was clipped, the [14C]phthalate diester was applied in a dose of 157 mumol/kg, and the area of application was covered with a perforated cap. The rat was restrained and housed for 7 days in a metabolic cage that allowed separate collection of urine and feces. Urine and feces were collected every 24 hr, and the amount of 14C excreted was taken as an index of the percutaneous absorption. At 24 hr, diethyl phthalate showed the greatest excretion (26%). As the length of the alkyl side chain increased, the amount of 14C excreted in the first 24 hr decreased significantly. The cumulative percentage dose excreted in 7 days was greatest for diethyl, dibutyl, and diisobutyl phthalate, about 50-60% of the applied 14C; and intermediate (20-40%) for dimethyl, benzyl butyl, and dihexyl phthalate. Urine was the major route of excretion of all phthalate diesters except for diisodecyl phthalate. This compound was poorly absorbed and showed almost no urinary excretion. After 7 days, the percentage dose for each phthalate that remained in the body was minimal and showed no specific tissue distribution. Most of the unexcreted dose remained in the area of application. These data show that the structure of the phthalate diester determines the degree of dermal absorption. Absorption maximized with diethyl phthalate and then decreased significantly as the alkyl side chain length increased. **(Also 3.4)**

Frasch, HF; Barbero, AM. (2005) **Application of solid-phase microextraction to in vitro skin permeation experiments: example using diethyl phthalate.** Toxicol In Vitro 19:253-259. The application of automated solid-phase microextraction (SPME) as a sample preparation technique for in vitro studies of skin permeation is described, using diethyl phthalate (DEP) as an example. In vitro diffusion cell experiments and skin-vehicle partition coefficient determinations require quantitative analysis of low-level analytes in aqueous samples. SPME is an ideal candidate for sample preparation for subsequent gas chromatographic analysis, offering numerous advantages over other methods. SPME conditions were optimized and the automated method was found to exhibit adequate sensitivity and good precision (relative standard deviation=3%). Abdominal skin (dermatomed at 350 microm) from male hairless guinea pigs (n=6) was used to measure DEP skin permeation parameters. In vitro methods were employed to determine permeability coefficient (k(p)), time lag (tau) and skin-buffer partition coefficient (K(SB)) for 2 mM DEP in HEPES buffered Hanks Balanced Salt Solution. Measurements (mean+/-standard deviations) are: k(p), 0.021+/-0.012 cm/h; tau, 0.67+/-0.18 h; K(SB), 4.74+/-0.68. The skin may be a significant route for the uptake of DEP.

Frasch, HF; Barbero, AM; Alachkar, H; et al. (2007) **Skin penetration and lag times of neat and aqueous diethyl phthalate, 1,2-dichloroethane and naphthalene.** Cutan Ocul Toxicol 26:147-160. Cutaneous exposures to occupational chemicals may cause toxic effects. For any chemical, the potential for systemic toxicity from dermal exposure depends on its ability to penetrate the skin. Most laboratory studies measure chemical penetration from an aqueous solution through isolated human or laboratory animal skin, although most exposures are not from pure aqueous solutions. The US EPA Interagency Testing Committee (ITC) mandated by the Toxic Substances Control Act, has required industry to measure the in vitro penetration of 34 chemicals in their pure or neat form (if liquid). The goal of the present study was to measure skin permeability and lag time for three neat chemicals of industrial importance, representing the general types of chemicals to be studied by the ITC (non-volatile liquids, volatile liquids, and solids), and to examine interlaboratory variation from these studies. Steady state fluxes and lag times of diethyl phthalate (DEP, slightly volatile), 1,2-dichloroethane (DCE, highly volatile), and naphthalene (NAP, solid) were studied in two different laboratories using different analytical methods. One lab also measured fluxes and lag times from saturated aqueous vehicle. Static diffusion cells, dermatomed hairless guinea pig skin, and gas chromatography were used to measure skin penetration. In the two laboratories, the steady state fluxes (mean+/-SD; microg cm(-2)hour(-1)) of DEP applied neat were: 11.8+/-4.1 and 23.9+/-7.0; fluxes of DCE (neat) were 6280+/-1380 and 3842+/-712; fluxes of NAP from powder were 30.4+/-2.0 and 7.5+/-4.7. Compared with neat fluxes measured in the same laboratory, flux from saturated aqueous solution was higher with DEP (1.9 x) but lower with DCE (0.17 x) and NAP (0.45 x). The three chemicals studied including a dry powder, demonstrate the potential for significant dermal penetration.

Mint, A; Hotchkiss SAM; Caldwell, J. (1994) **Percutaneous absorption of diethyl phthalate through rat and human skin in vitro.** Toxicology in Vitro 8:251-256. The percutaneous absorption of the plasticizer and fragrance chemical diethyl phthalate (DEP) has been evaluated in vitro in flow-through diffusion cells using shaved full-thickness skin from male Fischer 344 rats and human breast skin. Neat DEP (16.3-20.6 mg/cm2) was applied to the epidermal surface of the skin, which was then either left uncovered (unoccluded) or covered (occluded) with a teflon cap 2.9 cm above the skin surface. The absorption of DEP through rat skin and into the receptor fluid was relatively extensive reaching 35.9 +/- 2.9% (mean +/- SD, N = 4) of the applied dose over 72 hr when the skin was occluded and 38.4 +/- 2.5% (mean +/- SD, N = 3) when the skin was unoccluded. Absorption of DEP through human skin was significantly less (P less than 0.05) than through rat skin reaching 3.9 +/- 1.2% (mean +/- SD, N = 4) of the applied dose over 72 hr when the skin was occluded and 4.8 +/- 0.7% (mean +/- SD, N = 3) when the skin was unoccluded. Occlusion of the skin did not significantly alter the percutaneous absorption of DEP through rat or human skin. There was a four-fold variation in absorption between skin samples taken from human donors, ranging from 1.6 +/- 1.2% (mean +/- SD, N = 3) to 8.7 +/- 3.9% (mean +/- SD, N = 6) at 72 hr. This inter-individual variation was greater than the variation between animals, which ranged from 26.4 +/- 3.3% (mean +/- SD, N = 4) to 38.9 +/- 0.6% (mean +/- SD, N = 5). This information may be of significance for the safety evaluation of DEP for occupational and consumer use. Although human in vivo data are lacking, the percutaneous absorption of DEP through rat skin in vitro compares well with rat in vivo data from the literature, which supports the use of this technique as a model for in vivo absorption.

Scott, RC; Dugard, PH; Ramsey, JD; et al. (1987) **In vitro absorption of some o-phthalate diesters through human and rat skin.** Environ Health Perspect 74:223-227. The absorption of undiluted phthalate diesters [dimethyl phthalate (DMP), diethylphthalate (DEP), dibutyl phthalate (DBP) and di-(2-ethylhexyl)phthalate (DEHP)] has been measured in vitro through human and rat epidermal membranes. Epidermal membranes were set up in glass diffusion cells and their permeability to tritiated water measured to establish the integrity of the skin before the phthalate esters were applied to the epidermal surface. Absorption rates for each phthalate ester were determined and a second tritiated water permeability assessment made to quantify any irreversible alterations in barrier function due to contact with the esters. Rat skin was consistently more permeable to phthalate esters than the human skin. As the esters became more lipophilic and less hydrophilic, the rate of absorption was reduced. Contact with the esters caused little change in the barrier properties of human skin, but caused marked increases in the permeability to water of rat skin. Although differences were noted between species, the absolute rates of absorption measured indicate that the phthalate esters are slowly absorbed through both human and rat skin.

3.3. METABOLISM

Blount, BC; Silva, MJ; Caudill, SP; et al. (2000) **Levels of seven urinary phthalate metabolites in a human reference population.** Environ Health Perspect 108:979-982. Using a novel and highly selective technique, we measured monoester metabolites of seven commonly used phthalates in urine samples from a reference population of 289 adult humans. This analytical approach allowed us to directly measure the individual phthalate metabolites responsible for the animal reproductive and developmental toxicity while avoiding contamination from the ubiquitous parent compounds. The monoesters with the highest urinary levels found were monoethyl phthalate (95th percentile, 3,750 ppb, 2,610 microg/g creatinine), monobutyl phthalate (95th percentile, 294 ppb, 162 microg/g creatinine), and monobenzyl phthalate (95th percentile, 137 ppb, 92 microg/g creatinine), reflecting exposure to diethyl phthalate, dibutyl phthalate, and benzyl butyl phthalate. Women of reproductive age (20-40 years) were found to have significantly higher levels of monobutyl phthalate, a reproductive and developmental toxicant in rodents, than other age/gender groups ($p < 0.005$). Current scientific and regulatory attention on phthalates has focused almost exclusively on health risks from exposure to only two phthalates, di-(2-ethylhexyl) phthalate and di-isononyl phthalate. Our findings strongly suggest that health-risk assessments for phthalate exposure in humans should include diethyl, dibutyl, and benzyl butyl phthalates.

Lake, BG; Phillips, JC; Linnell, JC. (1977) **The in vitro hydrolysis of some phthalate diesters by hepatic and intestinal preparations from various species.** Toxicol Appl Pharmacol 39:239-248.

Rowland, IR; Cottrell, RC; Phillips, JC. (1977) **Hydrolysis of phthalate esters by the gastro-intestinal contents of the rat.** Food Cosmet Toxicol 15:17- 21. **(Also 4.5)**

3.4. ELIMINATION

Adibi, JJ; Perera, FP; Jedrychowski, W; et al. (2003) **Prenatal exposures to phthalates among women in New York City and Krakow, Poland.** Environ Health Perspect 111:1719-1722. Experimental evidence has shown that certain phthalates can disrupt endocrine function and induce reproductive and developmental toxicity. However, few data are available on the extent of human exposure to phthalates during pregnancy. As part of the research being conducted by the Columbia Center for Children's Environmental Health, we have measured levels of phthalates in 48-hr personal air samples collected from parallel cohorts of pregnant women in New York, New York, (n = 30) and in Krakow, Poland (n = 30). Spot urine samples were collected during the same 48-hr period from the New York women (n = 25). The following four phthalates or their metabolites were measured in both personal air and urine: diethyl phthalate (DEP), dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP), and butyl benzyl phthalate (BBzP). All were present in 100% of the air and urine samples. Ranges in personal air samples were as follows: DEP (0.26-7.12 microg/m3), DBP (0.11-14.76 microg/m3), DEHP (0.05-1.08 microg/m3), and BBzP (0.00-0.63 microg/m3). The mean personal air concentrations of DBP, di-isobutyl phthalate, and DEHP are higher in Krakow, whereas the mean personal air concentration of DEP is higher in New York. Statistically significant correlations between personal air and urinary levels were found for DEP and monoethyl phthalate ($r = 0.42$, $p < 0.05$), DBP and monobutyl phthalate ($r = 0.58$, $p < 0.01$), and BBzP and monobenzyl phthalate ($r = 0.65$, $p < 0.01$). These results demonstrate considerable phthalate exposures during pregnancy among women in these two cohorts and indicate that inhalation is an important route of exposure.

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

Beving, HF; Petren, S; Vesterberg, O. (1990) **Increased isotransferrin ratio and reduced erythrocyte and platelet volumes in blood from thermoplastic industry workers.** Ann Occup Hyg 34:391-397. Ten women (aged 31-61 years) and five men (aged 20-59 years) occupationally exposed to welding fumes of polyacetate containing diethylphthalate in a thermoplastic industry were studied. They had been employed 1-33 years (median: 11 years). Seven women (aged 35-55) and eight men (aged 26-73) acted as unexposed controls. The exposed persons showed increased isotransferrin ratio in blood serum and reduced volumes of erythrocytes and platelets in blood.

Calafat, AM; McKee, RH. (2006) **Integrating biomonitoring exposure data into the risk assessment process: phthalates [diethyl phthalate and di(2-ethylhexyl) phthalate] as a case study.** Environ Health Perspect 114:1783-1789. The probability of nonoccupational exposure to phthalates is high given their use in a vast range of consumables, including personal care products (e.g., perfumes, lotions, cosmetics), paints, industrial plastics, and certain medical devices and pharmaceuticals. Phthalates are of high interest because of their potential for human exposure and because animal toxicity studies suggest that some phthalates affect male reproductive development apparently via inhibition of androgen biosynthesis. In humans, phthalates are rapidly metabolized to their monoesters, which can be further transformed to oxidative products, conjugated, and eliminated. Phthalate metabolites have been used as biomarkers of exposure. Using urinary phthalate metabolite concentrations allows accurate assessments of human exposure because these concentrations represent an integrative measure of exposure to phthalates from multiple sources and routes. However, the health significance of this exposure is unknown. To link biomarker measurements to exposure, internal dose, or health outcome, additional information (e.g., toxicokinetics, inter- and intraindividual differences) is needed. We present a case study using diethyl phthalate and di(2- ethylhexyl) phthalate as examples to illustrate scientific approaches and their limitations, identify data gaps, and outline research needs for using biomonitoring data in the context of human health risk assessment, with an emphasis on exposure and dose. Although the vast and growing literature on phthalates research could not be covered comprehensively in this article, we made every attempt to include the most relevant publications as of the end of 2005.

Koo, HJ; Lee, BM. (2005) **Human monitoring of phthalates and risk assessment.** J Toxicol Environ Health A 68:1379-1392. Some phthalates, such as di(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate (DBP), and their metabolites are suspected of producing teratogenic and endocrino-disrupting effects. In this study, urinary levels of phthalates (DEHP, DBP, diethyl phthalate (DEP), butylbenzyl phthalate BBP), and monoethylhexyl phthalate (MEHP, a major metabolite of DEHP) were measured by high performance liquid chromatography (HPLC) in

human populations (women [hospital visitors], n = 150, and children, n = 150). Daily exposure level of DEHP in children was estimated to be 12.4 microg/kg body weight/d (male 9.9 microg/kg body weight/d, female 17.8 microg/kg body weight/d), but, in women was estimated to be 41.7 microg/kg body weight/d, which exceeded the tolerable daily intake (TDI, 37 microg/kg body weight/day) level established by the European Union (EU) Scientific Committee for Toxicity, Ecotoxicity, and the Environment (SCTEE) based on reproductive toxicity. Based on these data, hazard indices (HIs) were calculated to be 1.12 (41.7/37 TDI) for women and 0.33 (12.4/37 TDI) for children, respectively. These data suggest that Koreans (women and children) were exposed to significant levels of phthalates, which should be reduced to as low a level as technologically feasible to protect Koreans from the exposure to toxic phthalates

Marsee, K; Woodruff, TJ; Axelrad, DA; et al. (2006) **Estimated daily phthalate exposures in a population of mothers of male infants exhibiting reduced anogenital distance.** Environ Health Perspect 114:805-809. Phthalate diesters have been shown to be developmental and reproductive toxicants in animal studies. A recent epidemiologic study showed certain phthalates to be significantly associated with reduced anogenital distance in human male infants, the first evidence of subtle developmental effects in human male infants exposed prenatally to phthalates. We used two previously published methods to estimate the daily phthalate exposures for the four phthalates whose urinary metabolites were statistically significantly associated with developmental effects in the 214 mother-infant pairs [di-n-butyl phthalate (DnBP), diethyl phthalate (DEP), butylbenzyl phthalate (BBzP), diisobutyl phthalate (DiBP)] and for another important phthalate [di-2-ethylhexyl phthalate (DEHP)]. We estimated the median and 95th percentile of daily exposures to DBP to be 0.99 and 2.68 microg/kg/day, respectively; for DEP, 6.64 and 112.3 microg/kg/day; for BBzP, 0.50 and 2.47 microg/kg/day; and for DEHP, 1.32 and 9.32 microg/kg/day. The U.S. Environmental Protection Agency (EPA) reference doses for these chemicals are 100 (DBP), 800 (DEP), 200 (BBzP), and 20 (DEHP) microg/kg/day. The median and 95th percentile exposure estimates for the phthalates associated with reduced anogenital distance in the study population are substantially lower than current U.S. EPA reference doses for these chemicals and could be informative to any updates of the hazard assessments and risk assessments for these chemicals.

Silva, MJ; Barr, DB; Reidy, JA; et al. (2004) **Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000.** Environ Health Perspect 112:331-338. We measured the urinary monoester metabolites of seven commonly used phthalates in approximately 2,540 samples collected from participants of the National Health and Nutrition Examination Survey (NHANES), 1999-2000, who were greater than or equal to 6 years of age. We found detectable levels of metabolites monoethyl phthalate (MEP), monobutyl phthalate (MBP), monobenzyl phthalate (MBzP), and mono-(2-ethylhexyl) phthalate (MEHP) in > 75% of the samples, suggesting widespread exposure in the United States to diethyl phthalate, dibutyl phthalate or diisobutylphthalate, benzylbutyl phthalate, and di-(2-ethylhexyl) phthalate, respectively. We infrequently detected monoisononyl phthalate, mono-cyclohexyl phthalate, and mono-n-octyl phthalate, suggesting that human exposures to di-isononyl phthalate, dioctylphthalate, and dicyclohexyl phthalate, respectively, are lower than those listed above, or the pathways, routes of exposure, or pharmacokinetic factors such as absorption, distribution, metabolism, and elimination are different. Non-Hispanic blacks had significantly higher concentrations of MEP than did Mexican Americans and non-Hispanic whites. Compared with adolescents and adults, children had significantly higher levels of MBP, MBzP, and MEHP but had significantly lower concentrations of MEP. Females had significantly higher concentrations of MEP and MBzP than did males, but similar MEHP levels. Of particular interest, females of all ages had significantly higher concentrations of the reproductive toxicant MBP than did males of all ages; however, women of reproductive age (i.e., 20-39 years of age) had concentrations similar to adolescent girls and women 40 years of age. These population data on exposure to phthalates will serve an important role in public health by helping to set research priorities and by establishing a nationally representative baseline of exposure with which population levels can be compared.

4.2. LESS-THAN-LIFETIME AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

Anonymous. (8/75) **28-DAY SUBACUTE PERCUTANEOUS TOXICITY SL: VE-1005-20 SR: TGSE #1037 P75-006 WITH COVER LETTERS.** 878211859 OTS0206093 SUBCHRONIC TOXICITY, RABBITS, DERMAL

Brown, D; Butterworth, KR; Gaunt, IF; et al. (1978) **Short-term oral toxicity study of diethyl phthalate in the rat.** Food Cosmet Toxicol 16:415-422.

CONSUMER PROD TESTNG CO INC. (3/78) **FINAL REPORT, ACUTE DERMAL TOXICITY LD50 TEST OF DIETHYL PHTHALATE IN RATS WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000888S OTS0557298 ACUTE TOXICITY, RATS, DERMAL

CONSUMER PROD TSTNG CO INC. (3/78) **ORAL LD50 TEST IN RATS OF DIETHYL PHTHALATE WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000887S OTS0557297 ACUTE TOXICITY, RATS, GAVAGE

Food Research Laboratories, Inc.. (1955) **Toxicological studies of diethyl phthalate. Laboratory no. 67567..** Celanese Corp. of America, Summit Research Laboratories, Summit, NJ.

Foster PMD; Thomas, LV; Cook, MW. (1980) **Testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rat.** Toxicol Appl Pharmacol 54:392-398. **(Also 4.3)**

LEBERCO LABORATORIES. (2/78) **RABBIT EYE IRRITATION TEST OF DIETHYL PHTHALATE WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000894S OTS0557304 PRIMARY EYE IRRITATION, RABBITS, DERMAL

LEBERCO LABORATORIES. (9/63) **RABBIT EYE IRRITATION STUDY OF DIETHYL PHTHALATE WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000890S OTS0557300 PRIMARY EYE IRRITATION, RABBITS, DERMAL

Mapuskar, K; Pereira, C; Rao, CV. (2007) **Dose- dependent sub-chronic toxicity of diethyl phthalate in female Swiss mice.** Pestic Biochem Physiol 87:156-163. The experiment was conducted to study the after effects of administering DEP at different doses to female Swiss mice for a period of 90 days. Group I mice were fed on normal diet and water ad libitum. Group II mice were maintained on normal diet mixed with corn oil at 8.25mg/kg of the diet/day as oil control. Group III, IV and V mice were given diethyl phthalate dissolved in corn oil mixed with the diet at 10, 25 and 50mg/kg of the diet/day, which is approximately equal to 1.25, 3.125 and 6.25mg/kg body weight/day. A significant dose dependent increase was observed in serum acid phosphatase (ACP) whereas, serum and liver triglycerides levels showed a significant increase only in the high-dose treated group. Significant dose-dependent increase in serum aspartate and alanine aminotransferase (AST and ALT) and liver glycogen was observed. Serum lactate dehydrogenase (LDH) was significantly increased only in 25 and 50ppm DEP-treated mice. Liver cholesterol was significantly increased in all the treated groups. Liver histology by light microscopy showed intracellular vacuolations in all the treated groups which was much more evident in the 25 and 50ppm DEP-treated mice while hepatocellular degeneration and hypertrophy of the hepatocytes was evident in 50ppm DEP- treated mice. Proliferation of mitochondria and peroxisomes was evident in the electron micrographs of the 10ppm DEP-treated mice while 25 and 50ppm DEP-treated mice showed increase in lipid droplets and severe mitochondrial proliferation. **(Also 4.4, 4.6)**

Moody, DE; Reddy, JK. (1978) **Hepatic peroxisome (microbody) proliferation in rats fed plasticizers and related compounds.** Toxicol Appl Pharmacol 45:497-504. **(Also 4.6)**

NTP. (1995) **Toxicology and carcinogenesis studies of diethylphthalate (CAS No. 84-66-2) in F344/N rats and B6C3F1 mice (dermal studies) with dermal initiation/ promotion study of diethylphthalate and dimethylphthalate (CAS No. 131-11-3) in male Swiss (CD-1(R)) mi.** Natl Toxicol Program Tech Rep Ser 429:1-286. Diethylphthalate and dimethylphthalate are used as phthalate plasticizers, in an extensive array of products. The chronic dermal toxicity of diethylphthalate was evaluated in male and female F344/N rats and B6C3F1 mice in 2-year studies. In a series of special studies, the tumor initiation or promotion potential of diethylphthalate or dimethylphthalate was evaluated in male Swiss (CD-1) mice by an initiation/promotion model of skin carcinogenesis. The genetic toxicity of diethylphthalate and dimethylphthalate in Salmonella typhimurium and cultured Chinese hamster ovary cells was also evaluated. 4-WEEK STUDY IN F344/N RATS: Groups of 10 male

and 10 female rats were dermally administered diethylphthalate at volumes of 0, 37.5, 75, 150, or 300 mL (0, 46, 92, 184, or 369 mg) applied neat, 5 days per week for 4 weeks. All male and female rats survived to the end of the study. No evidence of dermatotoxicity was observed, with no adverse clinical signs observed and no effects on weight gain or feed consumption. Relative liver weights of 300 mL males and females and 150 mL females were greater than those of controls. Relative kidney weights of 150 and 300 mL males and 150 mL females were greater than those of controls. No other adverse effects were observed in this study. 4-WEEK STUDY IN B6C3F1 MICE: Groups of 10 male and 10 female mice were dermally administered diethylphthalate at volumes of 0, 12.5, 25, 50, or 100 mL (0, 15, 31, 62, or 123 mg) applied neat, five days per week for 4 weeks. One control female died before the end of the study; all other mice survived. No evidence of dermatotoxicity or other adverse clinical signs were observed, and no clear adverse effects on weight gain or feed consumption were seen. Absolute and relative liver weights of 25 and 100 mL females were greater than those of the controls. Based on these 4-week study results, doses of 0, 35, and 100 mL diethylphthalate were recommended for the 2-year mouse studies. A chronic study in male and female B6C3F1 mice at 0, 35, and 100 mL (applied neat, once per day, 5 days per week) was started and subsequently stopped after 32 weeks when significant body weight reductions were noted in treated animals (males and females, 100 mL groups: 19% lower; males, 35 mL group: 12% lower; females, 35 mL group: 10% lower than controls). Based on these body weight reductions, doses of 0, 7.5, 15, and 30 mL in 100 mL acetone were recommended for the restart of the 2-year mouse study. 2-YEAR STUDY IN F344/N RATS: Based upon the results of the 4-week study, doses of 0, 100, or 300 mL diethylphthalate (0, 123, or 369 mg) were chosen for the 2-year rat study. Groups of 60 male and 60 female rats received the doses applied neat 5 days per week for 103 weeks and up to 10 animals per group were evaluated after 15 months. Survival, Body Weights, and Clinical Findings: Survival of dosed rats during the first 15 months was similar to that of controls. However, 2-year survival was significantly reduced in all groups of male rats (survival probabilities, males: 0 mL, 8%; 100 mL, 12%; and 300 mL, 12%). The mean body weights of 300 mL males were slightly less than those of the controls throughout the study. No adverse clinical signs were observed, including no evidence of dermatotoxicity. Pathology Findings: No morphological evidence of dermal or systemic toxicity was observed in male or female rats. Skin neoplasms were not observed in female rats and were only rarely observed in male rats. A high incidence of anterior pituitary adenoma occurred in all groups of male and female rats. The incidence of anterior pituitary adenomas in the 0, 100, and 300 mL groups were: males, 39/44, 41/49, 41/49; females, 38/50, 33/49, 33/48. The incidence of this benign tumor in control males (84%) exceeded the historical control mean incidence [feed controls, (28.7%)] and range (12% to 60%). Anterior pituitary adenomas were considered a primary contributing factor in the increased mortality observed in all grtor in the increased mortality observed in all groups, regardless of treatment. A dose-related decreasing trend in the incidence of mammary gland fibroadenomas was observed in female rats (21/50, 12/48, 7/50). The incidence of mononuclear cell leukemia in male rats in this study was lower than the historical incidence and may be attributable to the shortened life span of male rats. Similarly, the incidence of interstitial cell tumors of the testes was markedly decreased in all groups of males (4/50, 3/50, 8/50), relative to historical control rates (90.1%; range 74%-98%). The incidence of fatty liver degeneration was notably lower in dosed rats than in controls (males: 26/50, 8/50, 4/51; females: 23/50, 11/50, 3/50). 2-YEAR STUDY IN B6C3F1 MICE: Groups of 60 male and 60 female mice received doses of 0, 7.5, 15, or 30 mL diethylphthalate (0, 9, 18, or 37 mg) in 100 mL acetone 5 days per week for 103 weeks with a 1 week recovery period, and up to 10 animals per group were evaluated after 15 months. Survival, Body Weights, and Clinical Findings: Two-year survival of dosed mice was similar to that of controls: 43/50, 41/48, 46/50, and 43/50 (males), and 41/50, 38/51, 37/49, and 36/49 (females). Mean body weights of dosed male and female mice were similar to those of the controls throughout the study. No adverse clinical signs were observed in mice, including no gross evidence of dermatotoxicity. Feed consumption by male and female mice was similar to or up to 13% greater than that by controls. Pathology Findings: No morphological evidence of dermal toxicity was observed in male or female mice. No skin neoplasms were observed in dosed male mice. In female mice receiving 30 mL, one squamous cell carcinoma and one basal cell carcinoma were seen at the site of application. An increased incidence of liver neoplasms was observed in dosed male and female mice. The incidence of hepatocellular adenoma or carcinoma (combined) in B6C3F1 mice in the 0, 7.5, 15, and 30 mL groups were: (males) 9/50, 14/50, 14/50, and 18/50; (females) 7/50, 16/51, 19/50, and 12/50. The incidence of adenoma or carcinoma (combined) was increased in 30 mL male mice and the incidences of adenoma and of adenoma or carcinoma (combined) were increased in 7.5 and 15 mL females. A positive dose-related trend in the incidence of adenoma or carcinoma (combined) was also observed in male mice. The incidence of basophilic hepatic foci was increased in 15 mL male mice (0/50, 1/50, 9/50, 3/50). The increased incidence of liver neoplasms in this study was considered equivocal because the incidence of hepatocellular neoplasms in control and dosed males was within the historical range and because there was no clear dose-response relationship in females. No other treatment-related findings were observed in this study. 1-YEAR INITIATION/PROMOTION STUDY IN MALE SWISS (CD-1) MICE: Groups of 50 male mice were

dosed dermally with diethylphthalate or dimethylphthalate to study their effect as initiators and promoters. Diethylphthalate and dimethylphthalate were tested as initiators with and without the known skin tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Diethyl phthalate and dimethylphthalate were tested as promoters with and without the known skin tumor initiator 7,12- dimethylbenzanthrancene (DMBA). Comparative control groups used during the study of diethylphthalate and dimethylphthalate included: vehicle control (acetone/acetone); initiation/promotion control (DMBA/TPA); initiator control (DMBA/acetone); and promoter control (acetone/TPA). Based on the incidence of skin neoplasms diagnosed histologically and the multiplicity of skin neoplasms, there was no suggestion that either diethylphthalate or dimethylphthalate was able to initiate skin carcinogenesis when chronically promoted by TPA. Further, there was no evidence that either diethylphthalate or dimethylphthalate was able to promote skin carcinogenesis in skin previously initiated with DMBA. High incidences of both squamous cell papillomas and squamous cell carcinomas occurred among the initiation/promotion control animals initiated with DMBA and promoted with TPA. All TPA-dosed groups had significantly greater incidences of dermal acanthosis, ulceration, exudation, and hyperkeratosis than controls. GENETIC TOXICOLOGY: Neither diethylphthalate (10-10,000 m/plate) nor dimethylphthalate (33-6,666 m/plate) induced gene mutations in Salmonella typhimurium strains TA98, TA100, TA1535, or TA1537, with or without rat and hamster liver S9. In cultured Chinese hamster ovary cells, both diethylphthalate and dimethylphthalate induced sister chromatid exchanges in the presence of S9. Neither induced sister chromatid exchanges in the absence of S9. Neither chemical induced chromosomal aberrations, with or without S9, in cultured Chinese hamster ovary cells. CONCLUSIONS: Under the conditions of these 2-year dermal studies, there was no evidence of carcinogenic activity of diethylphthalate in male or female F344/N rats receiving 100 or 300 mL. The sensitivity of the male rat study was reduced due to low survival in all groups. There was equivocal evidence of carcinogenic activity of diethylphthalate in male and female B6C3F1 mice based on increased incidences of hepatocellular neoplasms, primarily adenomas. In an initiation/promotion model of skin carcinogenesis, there was no evidence of initiating activity of diethylphthalate or dimethylphthalate in male Swiss (CD-1) mice. Further, there was no evidence of promotion activity of diethylphthalate or dimethylphthalate in male Swiss (CD-1) mice. The promoting activity of TPA following DMBA initiation was confirmed in these studies. Minor dermal acanthosis was observed following dermal application of diethylphthalate in male and female F344/N rats dosed for 2 years and in male Swiss (CD-1) mice dosed for 1 year. **(Also 4.4, 4.7)**

Pereira, C; Mapuskar, K; Rao, CV. (2006) **Chronic toxicity of diethyl phthalate in male Wistar rats--a dose-response study.** Regul Toxicol Pharmacol 45:169-177. Diethyl phthalate (DEP) is widely used in personal care products, plastics and medical devices at various concentrations, but its information is limited on its toxicity associated with exposure at high as well as low doses for a prolonged period. Therefore, a study was undertaken to understand the dose-response toxic effect of DEP in male Wistar rats. Control rats were fed on normal diet and water ad libitum. Rats were given DEP dissolved individually in corn oil mixed with the diet at 10, 25 and 50 mg/kg of the diet/day, which is equal to 0.57, 1.425 and 2.85 mg/kg body wt/day. After 5 months of treatment animals were sacrificed, enzymes and other biochemical parameters in the serum and liver were assessed. Liver weight to body weight ratio showed a significant increase only in 10 ppm DEP treated rats. A significant increase was observed in the serum ACP, LDH, ALT enzyme levels of 10 mg/kg treated rats as compared to control, 25 and 50 mg/kg treated rats. Other biochemical parameters like glycogen, total cholesterol, total triglycerides and lipid peroxidation were also increased in the liver of all the three treated groups. In the 10 and 50 mg/kg diet/day treated rats, there was a significant decrease in liver total GSH as compared to controls and 25 mg/kg treated rats. Histology of liver showed severe vacuolations, fatty degeneration and loss of hepatic architecture in the 10mg/kg treated rats, whereas in the 25 and 50 mg/kg treated rats only loss of hepatic architecture and granular deposits in the hepatocytes was predominant. Histology of liver by electron micrographs showed a significant dose- dependent proliferation of mitochondria in the hepatocytes, while the 10mg/kg treated rats showed increased number of peroxisomes in the hepatocytes. It is evident from this study that treatment with higher concentrations of DEP results in mitochondrial proliferation as well as accumulation of glycogen, cholesterol and triglycerides within the liver, but exposure to lower concentrations for longer periods results in increase in peroxisome numbers leading to severe hepatocellular changes which can be confirmed by significantly increased liver weights, elevated enzyme levels in the serum and liver and impaired metabolism of glycogen, cholesterol and triglyceride as well as altered liver histology. **(Also 4.6)**

Pereira, C; Rao, CV. (2006) **Combined and individual administration of diethyl phthalate and polychlorinated biphenyls and its toxicity in female Wistar rats.** Environ Toxicol Pharmacol 21:93-102. Polychlorinated biphenyls (PCBs) are persistent environmental pollutants and known to act as xenoestrogens. PCBs and diethyl phthalate (DEP) are ubiquitous environmental pollutants because both are used as plasticizers and in various other

industrial applications. Therefore, a study was undertaken to evaluate the interactive toxicity of DEP and PCBs in young female Wistar rats. Healthy young female albino rats of Wistar strain weighing 100 g (7-8 weeks old) were randomly assigned to five groups of six each. Group I female rats were fed on normal diet and water ad libitum. Group II female rats were maintained on normal diet mixed with corn oil at 16.5 mg/kg diet/day and 0.94 mg/kg body weight/day as oil control. Groups III and IV female rats were given Clophen A60 and DEP dissolved in corn oil mixed with the diet at 50 mg/(kg diet day), which is approximately equal to 2.85 mg/(kg body weight day), individually to each group. Group V female rats received a mixture of DEP and Clophen A60, each dissolved in corn oil mixed with the diet at 50 mg/(kg diet day), which is approximately equal to 2.85 mg/(kg body weight day). Treatment was carried out for 150 days and after the completion of treatment, serum and liver enzymes and other biochemical parameters in the serum and liver were assessed. Liver weight to body weight ratio showed significant increase in Clophen A60 and Clophen A60 + DEP treated rats. In the three treated groups, there was significant decrease in liver glutathione (GSH) and glutathione reductase (GR). Alanine amino transferase (ALT) was significantly increased in the liver of the three treated groups and in the serum of Clophen A60 and DEP alone treated groups and significant decrease only in the serum of Clophen A60 + DEP treated rats. Significant increase in liver and serum lactate dehydrogenase (LDH) and acid phosphatase (ACP) activity was observed in the three treated groups. Alkaline phosphatase (ALP) activity was significantly increased only in the serum of the Clophen A60 and Clophen A60 + DEP treated rats, whereas significant decrease in the serum and liver of DEP alone treated rats was observed. Aspartate aminotransferase (AST) activity and cholesterol levels were highly significant in the liver and serum of DEP treated rats. In addition, cholesterol level was significantly increased in the liver and serum of Clophen A60 treated rats and only in the liver of Clophen A60 + DEP treated rats. Succinate dehydrogenase (SDH) activity was significantly increased in the liver of Clophen A60 and Clophen A60 + DEP treated rats and highly significant increase in the serum of Clophen A60 + DEP treated rats. There was significant increase in triglyceride levels in the liver and serum of Clophen A60 and Clophen A60 + DEP treated rats, whereas significant increase in triglyceride levels in the serum of DEP alone treated rats was observed. Glycogen levels were significantly increased in the liver of Clophen A60 + DEP treated rats, whereas serum glucose levels showed significant decrease, but in Clophen A60 alone treated rats showed significant increase in liver glycogen and serum glucose, whereas DEP alone treated rats showed significant increase in only serum glucose levels. Lipid peroxidation was increased in the liver of DEP treated rats, which was highly significant, compared to significant increase in Clophen A60 and Clophen A60 + DEP treated rats. Histology of liver showed severe vacuolation, loss of hepatic architecture and granular deposits in the hepatocytes of DEP and Clophen A60 + DEP treated rats, whereas in Clophen A60 alone treated rats, hepatocytes showed hyper pigmentation mild loss of hepatic architecture in centrilobular and periportal area. **(Also 4.4, 4.6)**

SMYTH & SMYTH. (9/31) **INVESTIGATION OF TOXICITY OF CERTAIN PLASTICIZERS REPORT NO. 1. ACUTE TOXICITY TO SMALL ANIMALS.** 878211708 OTS0205855 RABBITS: In an acute toxicity study, rabbits (sex and strain not reported) were given single gavage exposures to diethyl phthalate and observed for 14 days. The animals were dosed at levels of 20, 10, 9, 8, 7.5, 7, 6, 5, 4, 2.5 or 1 g/kg which resulted in the following mortalities (number of deaths/number of animals dosed): 1/1, 3/4, 2/2, 3/3, 1/2, 2/3, 2/3, 1/4, 1/2, 0/1, and 0/1, respectively. Most of the deaths occurred within 24 hours. One 9 g/kg dose level animal died in 8 days and one 5 g/kg dose level animal died in 7 days. In an acute toxicity study, rabbits (sex and strain not reported) were given single intraperitoneal exposures to diethyl phthalate and observed for 14 days. The animals were dosed at levels of 10, 8, 6, 5, 4, 3, 2, 1 or 0.5 g/kg which resulted in the following mortalities (number of deaths/number of animals dosed): 1/1, 1/1, 3/3, 4/5, 2/4, 0/2, 0/2, 0/1, and 0/1, respectively. Most of the deaths occurred within 2 days. One 5 g/kg dose level animal died in 10 days. GUINEA PIGS: In an acute toxicity study, guinea pigs (sex and strain not reported) were given single gavage exposures to diethyl phthalate and observed for 14 days. The animals were dosed at levels of 10, 8, 7, 6, 5, 4, 3, 2, 1, 0.7 or 0.5 g/kg which resulted in the following mortalities (number of deaths/number of animals dosed): 2/3, 2/2, 2/2, 3/4, 1/6, 0/3, 0/2, 0/4, 0/2, 0/1, and 0/2, respectively. Most of the deaths occurred within 24 hours. One high-dose animal died in 8 hours and one 6 g/kg dose level animal died in 14 days. In an acute toxicity study, guinea pigs (sex and strain not reported) were given single intraperitoneal exposures to diethyl phthalate and observed for 14 days. The animals were dosed at levels of 5, 4, 3, 2.5, 2, 1 or 0.5 g/kg which resulted in the following mortalities (number of deaths/number of animals dosed): 2/2, 4/7, 3/8, 0/1, 5/8, 1/7, and 0/1, respectively. Most of the deaths occurred within 24 hours. One 2 g/kg animal died in 7 days and one 1 g/kg dose level animal died in 14 days. MICE: In an acute toxicity study, mice (sex and strain not reported) were given single intraperitoneal exposures to diethyl phthalate and observed for 14 days. The animals were dosed at levels of 10, 8, 6, 5, 4, 3, 2, 1, 0.8, 0.6 or 0.5 g/kg which resulted in the following mortalities (number of deaths/number of animals dosed): 1/1, 1/1, 1/1, 2/3, 5/5, 2/3, 0/5, 0/3, 0/1, 0/1, and 0/1, respectively. Most of the deaths occurred within 24

hours. One 6 g/kg animal died within 5 days and one 4 g/kg animal died within 4 days. RATS: In an acute toxicity study, rats (sex and strain not reported) were given single intraperitoneal exposures to diethyl phthalate and observed for 14 days. The animals were dosed at levels of 10, 8, 6, 5, 4, 3, 2, 1 or 0.5 g/kg which resulted in the following mortalities (number of deaths/number of animals dosed): 1/1, 1/1, 2/2, 4/4, 3/3, 3/3, 1/3, 2/3, and 0/1, respectively. Most of the deaths occurred within 24 hours, several animals in as little as 4 hours. One animal in each of the 6 and 2 g/kg dose level groups died within 7 days and 1 of the 1 g/kg animals died within 10 days. Several animals were prostrated within 1 hour of dosing.

Sonde, V; D'souza, A; Tarapore, R; et al. (2000) **Simultaneous administration of diethylphthalate and ethyl alcohol and its toxicity in male Sprague-Dawley rats.** Toxicology 147:23-31. Phthalate esters have been implicated as xenoestrogens. One among them is di-ethylphthalate (DEP), which is used as plasticizer, detergent base, and binder in incense sticks and after-shave lotions. DEP is one of the contaminants of freshwater and marine ecosystems. Incense stick workers are occupationally exposed to DEP and some workers are chronic alcoholics. Therefore, a study was undertaken to evaluate the interactive toxicity of DEP with ethyl alcohol (EtOH) in young male Sprague-Dawley rats. The rats were given 50 ppm DEP (w/v), 5% EtOH (v/v) and a combined dose of 50 ppm DEP (w/v)+EtOH (5% v/v) in water ad libitum for a period of 120 days and were maintained on normal diet. Control animals received normal diet and plain water. During the treatment rats were weighed every week and water consumption per day was measured. After the completion of treatment, liver weight/body weight, liver weight, body weight, serum enzymes and other biochemical parameters were assessed. It was found that there was no significant change observed in body weight, liver weight, liver weight/body weight and water consumption. It was observed that there was a significant decrease in liver aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in EtOH, DEP and EtOH+DEP treated rats in the order of EtOH>DEP>EtOH+DEP as compared with control. Serum AST, ALT, acid phosphatase (ACP), alkaline phosphatase (ALP), succinate dehydrogenase (SDH) and liver ACP showed significant increase in DEP and EtOH+DEP treated rats in the order of DEP>EtOH+DEP as compared with control and EtOH treated rats. On the contrary, there was no significant change in liver ALP levels in treated rats. There was significant increase in liver SDH, glycogen, total triglyceride, total cholesterol and lipid peroxidation in DEP and EtOH+DEP treated rats, but no significant changes in the serum SDH, glucose and total triglyceride levels. Serum total cholesterol levels in DEP and EtOH+DEP treated rats were significantly high as compared to control and EtOH treated rats. These results show that there is no interaction of DEP with EtOH but DEP alone leads to severe impairment of lipid metabolism coupled with toxic injury to the liver as evident from significantly altered lipid and enzyme levels in the liver and serum. Long term simultaneous exposure to DEP and EtOH may have severe implications for humans who are occupationally exposed to these two xenobiotics.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Anonymous. (1997) **Reproductive toxicology. Diethylphthalate.** Environ Health Perspect 105 Suppl 1:245-246. NOTE: summary of NTP study - see Lamb et al. 1987 for data results

Field, EA; Price, CJ; Sleet, RB; et al. (1993) **Developmental toxicity evaluation of diethyl and dimethyl phthalate in rats.** Teratology 48:33-44. Diethyl phthalate (DEP) and dimethyl phthalate (DMP), phthalic acid ester (PAE) plasticizers, were evaluated for developmental toxicity because of reports in the literature that some PAE were embryotoxic and teratogenic. A previous study (Singh et al., '72) suggested that an increased incidence of skeletal defects in rats might result from gestational exposure to DEP (0.6-1.9 g/kg) or DMP (0.4-1.3 g/kg), ip, on gestational days (gd) 5, 10, and 15. In the current study DEP (0, 0.25, 2.5, and 5%) or DMP (0, 0.25, 1, and 5%) in feed (approximately 0.2-4.0 g/kg/day) were supplied to timed-mated rats from gd 6 to 15. Treatment with 5% DMP resulted in increased relative maternal liver weight. Also, animals exhibited reduced body weight gain during treatment (5% DEP or DMP) and during gestation (5% DEP). Weight gain corrected for gravid uterine weight was also reduced in animals fed 5% DEP. However, high-dose treatment with either DEP or DMP resulted in changes in food and water consumption paralleling the body weight reductions, suggesting that apparent toxic effects on maternal body weight may reflect PAE/feed unpalatability. Treatment with 2.5% DEP resulted in only transient changes in body weight during early treatment. The only maternal effects at 0.25 or 1% DMP were minor changes in food and/or water consumption, and there were no effects at 0.25% DEP. Thus, the NOAELs for maternal toxicity were 1% DMP and 0.25% DEP. In contrast to the observed maternal toxicity, there was no effect of DEP or DMP treatment on any parameter of embryo/fetal development, except an increased incidence of supernumerary ribs (a variation) in the 5% DEP group. These results do not support the conclusion of other investigators that DEP and

DMP are potent developmental toxicants. Rather, they suggest that the short-chain PAE are less developmentally toxic than PAE with more complex substitution groups, e.g., di(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate, and butyl benzyl phthalate. **(Also 4.6)**

Foster, PM; Thomas, LV; Cook, MW; et al. (1983) **Effect of di-n-pentyl phthalate treatment on testicular steroidogenic enzymes and cytochrome P-450 in the rat.** Toxicol Lett 15:265-271. Treatment of young male rats with dipentyl phthalate (DPP) produced significant decreases in testicular cytochrome P-450, cytochrome P- 450 dependent microsomal steroidogenic enzymes (17 alpha-hydroxylase, 17-20 lyase) and in the maximal binding of a natural substrate (progesterone) to testis microsomes. No effect was demonstrated by this compound on hepatic cytochrome P-450 content. Treatment of animals with a phthalate ester not causing testicular atrophy (diethyl phthalate; DEP) produced no significant changes in any of the parameters measured. This effect on the enzymes responsible for androgen production may be important as a mechanism of action involved in the development of phthalate-induced testicular damage.

Fujii, S; Yabe, K; Furukawa, M; et al. (2005) **A two- generation reproductive toxicity study of diethyl phthalate (DEP) in rats.** J Toxicol Sci 30 Spec No.:97- 116. A two-generation reproductive toxicity study was performed to evaluate the effects of diethyl phthalate on parental reproductive performance, including features of the endocrine system and development and growth of offspring at dietary dose levels of 0, 600, 3000 and 15000 ppm. In F0 and F1 parents, no treatment-related adverse effects were observed in clinical findings, body weights, food consumption, reproductive parameters, and gross or histopathological findings in any treated group. Increased liver weights and enhanced activities of metabolic enzymes were observed in F0 males at 15000 ppm. F0 males also exhibited an increase in the content of CYP3A2, a cytochrome P450 isozyme, at 15000 ppm, and a decrease in the levels of serum testosterone at 3000 and 15000 ppm, suggesting sex steroid metabolism might be changed. However, these were not considered adverse effects because the degree of change was too slight to affect the reproductive capability to produce progeny. Body weight gains before weaning were inhibited in F1 and F2 pups and vaginal opening was slightly delayed in F1 females at 15000 ppm. No changes were observed in the reproductive performance. Therefore, the no-observed-adverse-effect level (NOAEL) from this study is considered to be 15000 ppm for parental animals, and 3000 ppm for development and growth of the pups. **(Also 4.6)**

Gray, LE; Ostby, J; Furr, J; et al. (2000) **Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat.** Toxicol Sci 58:350-365. In mammals, exposure to antiandrogenic chemicals during sexual differentiation can produce malformations of the reproductive tract. Perinatal administration of AR antagonists like vinclozolin and procymidone or chemicals like di(2-ethylhexyl) phthalate (DEHP) that inhibit fetal testicular testosterone production demasculinize the males such that they display reduced anogenital distance (AGD), retained nipples, cleft phallus with hypospadias, undescended testes, a vaginal pouch, epididymal agenesis, and small to absent sex accessory glands as adults. In addition to DEHP, di-n-butyl (DBP) also has been shown to display antiandrogenic activity and induce malformations in male rats. In the current investigation, we examined several phthalate esters to determine if they altered sexual differentiation in an antiandrogenic manner. We hypothesized that the phthalate esters that altered testis function in the pubertal male rat would also alter testis function in the fetal male and produce malformations of androgen-dependent tissues. In this regard, we expected that benzyl butyl (BBP) and diethylhexyl (DEHP) phthalate would alter sexual differentiation, while dioctyl tere- (DOTP or DEHT), diethyl (DEP), and dimethyl (DMP) phthalate would not. We expected that the phthalate mixture diisononyl phthalate (DINP) would be weakly active due to the presence of some phthalates with a 6-7 ester group. DEHP, BBP, DINP, DEP, DMP, or DOTP were administered orally to the dam at 0.75 g/kg from gestational day (GD) 14 to postnatal day (PND) 3. None of the treatments induced overt maternal toxicity or reduced litter sizes. While only DEHP treatment reduced maternal weight gain during the entire dosing period by about 15 g, both DEHP and DINP reduced pregnancy weight gain to GD 21 by 24 g and 14 g, respectively. DEHP and BBP treatments reduced pup weight at birth (15%). Male (but not female) pups from the DEHP and BBP groups displayed shortened AGDs (about 30%) and reduced testis weights (about 35%). As infants, males in the DEHP, BBP, and DINP groups displayed femalelike areolas/nipples (87, 70, and 22% (p less than 0.01), respectively, versus 0% in other groups). All three of the phthalate treatments that induced areolas also induced a significant incidence of reproductive malformations. The percentages of males with malformations were 82% (p less than 0.0001) for DEHP, 84% (p less than 0.0001) for BBP, and 7.7% (p less than 0.04) in the DINP group. In summary, DEHP, BBP, and DINP all altered sexual differentiation, whereas DOTP, DEP, and DMP

were ineffective at this dose. Whereas DEHP and BBP were of equivalent potency, DINP was about an order of magnitude less active.

Gray, TJ; Butterworth, KR. (1980) **Testicular atrophy produced by phthalate esters.** Arch Toxicol Suppl 4:452-455. In a 90-day toxicity study in rats, di-(2-ethylhexyl)phthalate (DEHP) produced testicular atrophy. To characterise further the testicular toxicity of phthalate esters the effect of age on the induction of testicular atrophy has been examined as well as the reversibility of the lesions and the effects of certain other phthalate esters. Seminiferous tubular atrophy, comprising a loss of spermatids and spermatocytes, resulted when 4-week-old rats were given 10 daily doses of DEHP. In similarly treated 10-week old rats up to 50% of tubules were atrophic while the remainder were unaffected. No testicular damage was produced in 15 week-old rats. The lesion produced in 4-week-old rats was reversible whether treatment was stopped prior to, or continued until after the control rats had reached sexual maturity. Normal testicular weight and histology were restored within 12 and 20 weeks respectively. Of a series of di-n-alkyl phthalates from dimethyl to di-n-octyl, the butyl, pentyl and hexyl esters produced testicular lesions similar to DEHP. The testicular effects of DEHP were not influenced by simultaneous administration of testosterone or follicle stimulating hormone (FSH). The mechanism by which phthalate esters exert their effects is discussed in the context of a possible action on Sertoli cell function.

Hardin, BD; Schuler, BD; Burg, RL. (1987) **Evaluation of 60 chemicals in a preliminary developmental toxicity test.** Teratog Carcinog Mutagen 7:29-48. The number of chemicals in commerce which have not been evaluated for potential developmental toxicity is large. Because of the time and expense required by conventional developmental toxicity tests, an abbreviated assay is needed that will preliminarily evaluate otherwise untested chemicals to help prioritize them for conventional testing. A proposed short-term in vivo assay has been used in a series of studies in which a total of 60 chemicals were tested. Some were independently tested two or four times each. In this preliminary test, pregnant mice were dosed during mid-pregnancy and were then allowed to deliver litters. Litter size, birth weight, and neonatal growth and survival to postnatal day 3 were recorded as indices of potential developmental toxicity. Results in this assay and conventional mouse teratology tests were generally concordant. Conventional data were available for 14 chemicals (ten teratogens, one fetotoxin, three nonteratogens), of which 11 (nine teratogens, one fetotoxin, one nonteratogen) produced evidence of developmental toxicity. This included conventional data for three chemicals (ethylene glycol, diethylene glycol dimethyl ether, and triethylene glycol dimethyl ether) that were untested before the present study. As high priority candidates for conventional testing on the basis of results here, all were subsequently studied in a standard teratology assay and were confirmed to be teratogenic in mice. Additionally, one of them (ethylene glycol) plus a fourth high priority candidate for conventional study (diethylene glycol monomethyl ether) were subsequently tested in rats and were found to be teratogenic in that species.

HAZLETON LABORATORIES. (12/8) **INITIAL SUBMISSION: SCREENING OF PRIORITY CHEMICALS FOR POTENTIAL REPRODUCTIVE HAZARD (FINAL REPORT) WITH COVER LETTER DATED 041492.** 88-920002015 OTS0536209 Diethyl phthalate (84-66-2) was administered by gavage to groups of 50 Charles River CD-1 pregnant female mice at dose levels of 0 or 4,500 mg/kg/day for 3 hours/day on days 7-14 of gestation. Animals were observed for clinical signs of toxicity. One mouse exposed to diethyl phthalate died on day 8 of gestation (treatment day 2) and one mouse was sacrificed in moribund condition. One mouse aborted on day 3 of gestation. Surviving animals were allowed to deliver and care for their young (number of live pups, dead pups, and total litter weight were measured on lactation days 1 and 3). Mean body weights and body weight changes, number of pregnancies, number of pups per litter, and number of viable litters were not significantly different from control data (p less than 0.05).

HAZLETON LABORATORIES. (12/8) **SCREENING OF PRIORITY CHEMICALS FOR POTENTIAL REPRODUCTIVE HAZARD (FINAL REPORT) WITH ATTACHMENTS AND COVER SHEET.** 86-870001624 OTS0516205 TERATOGENICITY, MICE, GAVAGE

Jones, HB; Garside, DA; Liu, R; et al. (1993) **The influence of phthalate esters on Leydig cell structure and function in vitro and in vivo.** Exp Mol Pathol 58:179-193. Phthalate esters are widely used in the manufacture of plastics and have been shown to cause testicular toxicity, purportedly, by targeting the Sertoli cell alone. Recent evidence, however, indicates that a paracrine control exists between Sertoli and Leydig cells and the breakdown of one component of this relationship is therefore detrimental to normal function. However, no data that explore the

influence of testicular toxins on Leydig cell structure and function have been published hitherto. The preliminary studies reported here were initiated to test the hypothesis that phthalate intoxication may adversely alter Leydig cell structural and functional integrity. Four phthalate esters, namely, di(2-ethylhexyl) phthalate (DEHP, di-n-pentyl phthalate (DPP)., di-n-octyl phthalate (DOP), and diethyl phthalate (DEP) were investigated in vivo and their monoesters (MEHP, MPP, MOP, and MEP, respectively) in vitro for indications of Leydig cell toxicity in the rat. Rats were dosed by oral gavage with 2 g phthalate diester/kg/day in corn oil vehicle for 2 days, while Leydig cell primary cultures were incubated with 1,000 microM monoester for 2 hr. Light and electron microscopy were undertaken to determine the type and degree of any changes. Phthalate esters exerted a direct effect on Leydig cell structure and function (as determined by testosterone output) with correlation of the in vitro and in vivo effects of MEHP (DEHP) and MOP (DOP). No effects on Leydig cell structure or function were seen with MPP (DPP), although Sertoli cell cytoplasmic rarefaction and vacuolation were observed in vivo. DEP produced Leydig cell ultrastructural alterations in vivo. We conclude that individual phthalate esters may exert effects on both Sertoli and Leydig cells or one cell type alone. **(Also 4.6)**

Lamb, JC; Chapin, RE; Teague, J; et al. (1987) **Reproductive effects of four phthalic acid esters in the mouse.** Toxicol Appl Pharmacol 88:255-269. These studies compared the reproductive toxicity of four phthalates by a continuous breeding protocol. Mice were given diets with diethyl phthalate (DEP) (0.0, 0.25, 1.25, or 2.5%), di-n-butyl phthalate (DBP) (0.0, 0.03, 0.3, or 1.0%), di-n-hexyl phthalate (DHP) (0.0, 0.3, 0.6, or 1.2%), or di(2-ethylhexyl) phthalate (DEHP) (0.0, 0.01, 0.1, or 0.3%). Both male and female CD-1 mice were dosed for 7 days prior to and during a 98-day cohabitation period. Reproductive function was evaluated during the cohabitation period by measuring the numbers of litters per pair and of live pups per litter, pup weight, and offspring survival. There was no apparent effect on reproductive function in the animals exposed to DEP, despite significant effects on body weight gain and liver weight. DBP exposure resulted in a reduction in the numbers of litters per pair and of live pups per litter and in the proportion of pups born alive at the 1.0% amount, but not at lower dose levels. A crossover mating trial demonstrated that female mice, but not males, were affected by DBP, as shown by significant decreases in the percentage of fertile pairs, the number of live pups per litter, the proportion of pups born alive, and live pup weight. DHP in the diet resulted in dose-related adverse effects on the numbers of litters per pair and of live pups per litter and proportion of pups born alive at 0.3, 0.6, and 1.2% DHP in the diet. A crossover mating study demonstrated that both sexes were affected. DEHP (at 0.1 and 0.3%) caused dose-dependent decreases in fertility and in the number and the proportion of pups born alive. A crossover mating trial showed that both sexes were affected by exposure to DEHP. These data demonstrate the ability of the continuous breeding protocol to discriminate the qualitative and quantitative reproductive effects of the more and less active congeners as well as the large differences in reproductive toxicity attributable to subtle changes in the alkyl substitution of phthalate esters. **(Also 4.6)**

NTP. (1984) **Diethyl phthalate (CAS no. 84-66-2): Reproduction and fertility assessment in CD-1 mice when administered in the feed.** Natl Toxicol Program Tech Rep Ser; Report RACB83092. Diethylphthalate (DEP) was tested for reproductive toxicity in the standard Continuous Breeding design using Swiss CD-1 mice. Data collected in the Task 1 dose-range-finding study (body weight, clinical signs, food and water consumptions) were used to select concentrations of 0.0, 0.25, 1.25, and 2.5% in feed for the main study. Feed consumption was not affected by the presence of DEP. These concentrations in feed gave calculated consumption estimates of 0.34, 1.77, and 3.64 g DEP/kg body weight/day. DEP consumption had no adverse effect on the mean number of litters/pair, the number of live pups/litter, the viability of the pups, or pup body weight adjusted for litter size. In fact, the number of pups/litter were increased at the low and middle doses by 32% and 14%, respectively. We attribute this to the fact that the value for the controls was nearly equal to 25% below historical values for this strain. Because there was no observable change in fertility or reproductive outcome, Task 3 (the crossover mating trial to determine the affected sex) was not conducted. The second generation was tested using the F1 mice from the control and high dose groups. There was no difference between these two groups in terms of body weights or viability of the F1 pups at birth, weaning, or at the start of the week of mating (pnd 74 ± 10). All 20 pairs of mice mated in both groups, and 95% of those delivered live young (in both groups). The DEP litters had 14% fewer pups; viability and pup weight adjusted for litter size were unchanged. After the F2 pups were evaluated and discarded, the F1 adults were killed and necropsied. Treated females weighed 8% less, while adjusted liver weight was increased by 28%. Treated males weighed 12% less than their respective controls, while their liver weight and prostate weight, both adjusted for body weight, were increased by 18% and 32%, respectively. Epididymal sperm concentration was reduced by 30%, while the percent motile sperm and the proportion of abnormal forms were unaffected by DEP. In summary, DEP had no

effect on F0 reproductive performance, while producing moderate reproductive effects in the second generation in the presence of mild body weight gain inhibitions and moderate increases in liver weight. **(Also 4.6)**

NTP. (1988) **Developmental toxicity of diethyl phthalate (CAS no. 84-66-2) administered to CD rats on gestational days 6 through 15.** Natl Toxicol Program Tech Rep Ser; Report TER88065. Diethyl phthalate (DEP), a member of the phthalic acid ester class of plasticizers, was evaluated for toxic and teratogenic effects in timed-pregnant CD rats (n=27-32 confirmed pregnancies/group). Dietary concentrations of 0, 0.25, 2.5, and 5.0% DEP were administered on gestational days (gd) 6 through 15, with resulting average doses of 198, 1909 and 3214 mg/kg/day for the low-, mid- and high-concentration groups, respectively. Females were weighed and observed daily during treatment for clinical signs. Maternal food and water consumption was measured throughout gestation. At sacrifice on gd 20, maternal liver and kidneys were weighed. In addition, the uterus of each dam was weighed, and the number and status of uterine implantation sites were recorded. Each live fetus was weighed, sexed, and examined for external, visceral, and skeletal malformations. No adverse effects upon maternal status were observed at 0.25% DEP in the diet; this group exhibited increased body weight (gd 20), weight gain (gd 6-15 and gd 0-20), and corrected weight gain relative to controls. Transient maternal toxicity in the form of reduced body weight after three days of exposure (gd 9) was observed in the 2.5% dose group with recovery by gd 12. Reduction in maternal body weight gain was observed in the 5. O% dose group during the treatment period (gd 6-15), and in body weight on gd 9, 12, 15, and 18. Maternal food and water consumption (both absolute and relative) were depressed between gd 6-9 (after initiation of dosing on gd 6) in the 2.5% DEP and 5.0% DEP groups. Depression of food intake at 5.0% DEP continued during gd 9-12. A rebound increase in relative food consumption (g/kg/d) was noted between gd 12-15 at 2.5% DEP and between gd 12-15, 15- 18, and 18-20 at 5.0% DEP. No adverse effects upon embryo/fetal growth, viability or the incidence of malformations were observed even at 5 0% DEP in the feed The incidence of extra rib, classified as a variation in this species and strain, was significantly increased at 5 .0% DEP in the feed. In conclusion, DEP administered to pregnant CD rats during the period of major organogenesis had no adverse effect upon embryo/fetal development except for an increased incidence in extra rib (an anatomical variation) at a maternally toxic exposure level. **(Also 4.6)**

Oishi, S; Hiraga, K. (1980) **Testicular atrophy induced by phthalic acid esters: Effect on testosterone and zinc concentrations.** Toxicol Appl Pharmacol 53:35-41. **(Also 4.6)**

Pereira, C; Mapuskar, K; Vaman, RC. (2007) **A two- generation chronic mixture toxicity study of Clophen A60 and diethyl phthalate on histology of adrenal cortex and thyroid of rats.** Acta Histochem 109:29-36. This study was undertaken to observe the type of interaction that exists between polychlorinated biphenyls (Clophen A60) and diethyl phthalate (DEP) on the adrenal and thyroid glands of male and female Wistar rats. Animals were divided into four groups of six animals each, group I male and female rats were fed on a normal diet and water ad libitum. Groups II, III and IV male and female rats were given Clophen A60, DEP, or mixture of Clophen A60 and DEP, respectively, each dissolved in corn oil mixed with the diet at 50mg/kg of the diet/day. One hundred days after treatment, females were mated with males for 10 days. Exposure to the pollutants was continued throughout mating, gestation (21 days) until termination at weaning (21 days), which was 150 days of total treatment period of the parental generation. When the F1-generation pups (six males and six females of each group) were 75-100g in weight, they were treated in a similar manner to the parental generation, again for a period of 150 days, with the dose reduced to 25mg/kg of the diet/day in all treated groups. After 150 days of treatment, animals were sacrificed and histology of the adrenal and thyroid glands was asessed. An antagonistic interactive effect of treatment was seen in male parental and F1-generation rats, while an inhibitory type of interactive effect was observed in female rats. In the zona fasciculata region of the adrenal cortex of treated rats of both generations, vacuolations and degeneration were seen in samples from male animals and intracellular vacuolations in samples from females. A synergistic interactive toxic effect to the thyroid gland was observed in treated parental generation male rats, and mild changes in F1-generation-treated male rats, showing follicular shrinkage, loss of thyroglobulin and fibrosis of the interfollicular epithelium. In females, an antagonistic effect to the thyroid gland was observed in both parental and F1-generation-treated rats, showing similar effects as observed in males. From this study, we can conclude that combined administration of Clophen A60 and DEP shows an enhanced toxic effect on adrenal glands of F1-generation male and female rats, but the effect is much more marked in the thyroid gland of F1-generation male rats, and seen to a lesser extent in F1-generation female rats. **(Also 4.4, 4.6)**

Pereira, C; Mapuskar, K; VamanRao, C. (2007) **Chronic toxicity of diethyl phthalate--A three generation lactational and gestational exposure study on male Wistar rats.** Environmental Toxicology and Pharmacology 23:319-327. Diethyl phthalate (DEP) is widely used in the perfume industry as a vehicle for fragrances and in personal care products making human exposure of DEP significant to adults as well as neonatals, as confirmed by levels recorded in blood as well as breast milk samples of human populations in some parts of the world. Therefore, a study was undertaken to understand the toxic effect of DEP over three generations in male Wistar rats. Healthy male and female albino rats of Wistar strain weighing 75-100 g (6-7 weeks old) were randomly assigned to two groups of six each. Group I (Control) male and female rats were fed on normal diet and water ad libitum. Group II (DEP) male and female rats were given DEP dissolved in corn oil mixed with the diet at 50 mg/kg of the diet/day. Hundred days after the treatment, females were mated with males for 10 days. Exposure to DEP was continued throughout mating, gestation until termination at weaning, which was 150 days of total treatment period of the parental generation. The F1 and F2 generation pups were then segregated on the basis of their sex and six male and female pups of both generations were allowed to grow till they were 75-100 g in weight. The treatment was then carried out similar to the parental generation but with reduced dose of 25 mg/kg of the diet/day for F1 generation and 10 mg/kg of the diet/day for F2 generation. Hundred days after the treatment, females were mated with males for 10 days. Exposure to DEP was continued throughout mating, gestation (21 days) until termination at weaning (21 days), which was 150 days of total treatment period of the F1 and F2 generation. Liver and serum ALT, AST and triglycerides were significantly increased over the three generations, which was much more significant in the F2 generation DEP treated group. The serum cholesterol and liver glutathione and glutathione reductase showed a significant decrease over the three generations, which was much more significant in the F2 generation DEP treated group as compared to the parental and F1 generation DEP treated rats. Histology of the liver showed remarkably enhanced fatty degeneration in the F2 generation DEP treated rats as compared to parental and F1 generation DEP treated rats. Vacuolations were much more significant in the F1 generation DEP treated rats as compared to the controls and F2 generation DEP treated rats. It can be concluded from this study, that continuous exposure through food, gestation and lactation over three generation's inspite of dose reduction of DEP leads to an enhanced toxic effect in the latter generations. **(Also 4.6)**

Pereira, C; Rao, CV. (2007) **Toxicity study of maternal transfer of polychlorinated biphenyls and diethyl phthalate to 21-day-old male and female weanling pups of Wistar rats.** Ecotoxicol Environ Saf 68:118-125. Polychlorinated biphenyls (PCBs) are environmental pollutants known to act as xenoestrogens. PCBs and diethylphthalate (DEP) are ubiquitous environmental pollutants because both are used as plasticizers and in various other industrial applications. Therefore, a study was undertaken to evaluate the interactive toxicity of DEP and PCB in 21-day-old male and female pups of Wistar rats. Healthy young male and female albino rats of Wistar strain weighing 75-100g (6-7 weeks old) were randomly assigned to four groups of six each. Group I male and female rats were fed a normal diet and water ad libitum. Group II and III male and female rats were given PCB (Clophen A60) and DEP dissolved in corn oil mixed with the diet at 50mg/kg of the diet (2.85mg/kg body wt) individually to each group. Group IV male and female rats received a mixture of DEP and PCB (Clophen A60), each dissolved in corn oil mixed with the diet at 50mg/kg of the diet (2.85mg/kg body wt). Hundred days after the treatment, females were mated with males for 10 days. Exposure to DEP and PCB was continued throughout mating, gestation until termination at weaning, which was 150 days of total treatment period of adults. The pups from each group were then segregated on the basis of their sex. Six male and female pups each (approx. 21 days old) from each group were chosen randomly and were killed for toxicity study. Liver-to-body weight ratio showed significant increase in the male and female pups of PCB- and PCB+DEP-treated rats, whereas male pups of DEP alone treated rats showed significant increase and female pups showed significant decrease as compared to controls and other treated groups. Significant increase in liver and serum lactate dehydrogenase (LDH) and acid phosphatase (ACP) activity in the male and female pups of the three treated groups was observed. Alkaline phosphatase (ALP) activity was significantly increased only in the serum of male and female pups of the three treated groups, whereas significant decrease in the liver of male pups of the three treated groups. In the female pups, significant decrease in liver ALP was observed only PCB- and PCB+DEP-treated groups. Histology of liver showed severe vacuolation and steatosis in the hepatocytes of PCB-treated male and female pups and in PCB+DEP-treated group, vacuolation, and steatosis was much more predominant as compared to the PCB and DEP alone treated groups. DEP alone treated groups, both male and female pups showed mild vacuolations in the liver. A synergistic interactive toxic effect of PCB and DEP was evident in both male and female pups in the following study. **(Also 4.4, 4.6)**

PROCTER & GAMBLE CO. (12/8) **TERATOGENICITY STUDY OF E-2426.01 (DIETHYL PHTHALATE) BY DERMAL APPLICATION TO RABBITS WITH COVER LETTER DATED 05/02/94.** 86940000362 OTS0572465 TERATOGENICITY, RABBITS, DERMAL

Singh, AR; Lawrence, WH; Autian, J. (1972) **Teratogenicity of phthalate esters in rats.** J Pharmacol Sci 61:51-55.

UNIV TENN. (3/71) **TERATOGENICITY OF A GROUP OF PHTHALATE ESTERS IN RATS.** 878213812 OTS0206451 TERATOGENICITY, RATS, INTRAPERITONEAL

Yamasaki, K; Takahashi, M; Yasuda, M. (2005) **Two-generation reproductive toxicity studies in rats with extra parameters for detecting endocrine disrupting activity: introductory overview of results for nine chemicals.** J Toxicol Sci 30 Spec No.:1-4. Two-generation reproductive toxicity studies using rats of benzophenone, n-butylbenzene, butyl benzyl phthalate, 2,4-dichlorophenol, dicychlohexyl phthalate, diethyl phthalate, 4- nitrotoluene, lindane and vinclozolin, were performed to investigate whether these chemicals have endocrine-mediated effects with the support of the Ministry of Economy, Trade and Industry and the Ministry of the Environment. Benzophenone exposure was via the diet at concentrations of 0, 100, 450 or 2000 ppm, n-butylbenzene was administered orally by gavage at dose levels of 0, 30, 100 or 300 mg/kg/day, butyl benzyl phthalate was administered orally by gavage at dose levels of 0, 100, 200, or 400 mg/kg/day, 2,4-dichlorophenol was administered in the diet at concentrations of 0, 500, 2000 or 8000 ppm, dicyclohexyl phthalate was given in the diet at concentrations of 0, 240, 1200 or 6000 ppm, diethyl phthalate was administered in the diet at concentrations of 0, 600, 3000 or 15000 ppm, 4-nitrotoluene was administered orally by gavage at doses of 0, 40, 80, or 160 mg/kg/day, lindane exposure was in the diet at concentrations of 0, 10, 60, or 300 ppm, and vinclozolin treatment was by feeding diet at concentrations of 0, 40, 200 or 1000 ppm. NOTE: see Fujii et al. 2005 for detailed results of DEP study.

4.4. OTHER ENDPOINT-SPECIFIC STUDIES

Anonymous. (1984) **Results of skin irritation tests on diethyl phthalate.** EPA/OTS Doc #878214848

Api, AM. (1997) **In vitro assessment of phototoxicity.** In Vitro Toxicol 10:339-350. A major criticism of many of the in vitro phototoxicity assays currently under development is that they have been used to test only water-soluble materials. In this study, seven water-insoluble materials were selected for testing in an in vitro phototoxicity assay using a human skin model, Skin super(2). The results show that 8-methoxypsoralen, isopsoralen, 7-methoxycoumarin, and methyl-n-methylanthranilate were phototoxic when evaluated in this test system. Coumarin, at concentrations up to 5%, was not phototoxic. These results are the same as those found in vivo in animals and humans. At higher concentrations, musk ambrette was phototoxic; however, these observations were not confirmed when the experiment was repeated. In humans, musk ambrette is a photoallergen, but not phototoxic. Thus this in vitro test method accurately identifies the materials that were found to be phototoxic in humans. Experiments conducted with undiluted diethyl phthalate show that the material is toxic to the Skin super(2) tissue. It was possible to test the undiluted material in this assay at an exposure time of 1 hour rather than 16 to 24 hours, but a different exposure time would require extensive testing to validate this modification of the test method.

Api, AM. (2002) **Sensitization methodology and primary prevention of the research institute for fragrance materials.** Dermatology 205:84-87. The Research Institute for Fragrance Materials Inc. (RIFM) has approached sensitization studies with fragrance materials as primary prevention of sensitization in the healthy, normal population. Secondary prevention, or avoidance of elicitation, most often suggested by dermatologists for patients presenting with dermatitis, has not been part of its program effort. Historically, RIFM evaluated the sensitization potential of fragrance materials using the human maximization test method; no animal models were used. In general, petrolatum was used as the vehicle. This is a harsh procedure whose main use may provide a measure of the uppermost limits of sensitization. Treating skin with sodium lauryl sulfate may be problematic and finding a laboratory to conduct the study may also be difficult. In addition, using a human predictive test method for both hazard and safety assessments is not ideal. The current practice involves a hazard assessment using an animal model, followed by a safety assessment in a human repeated-insult patch test (HRIPT). The animal test method is used to identify the sensitization potential and a no-effect level. Following a review of the no-effect level and the maximum

skin level, a safety assessment in humans can be conducted. RIFM also modified the original vehicle used in sensitization testing, since petrolatum presents two major difficulties: solubility and inconsistent effects on skin penetration. Since the greatest exposure to fragrance materials is considered to be from a cologne- type product, ethanol was chosen as a more realistic vehicle. Further modification resulted in combinations of ethanol and diethyl phthalate, due to diethyl phthalate's use in many perfume formulations as a solvent and fluidizer. Human testing should not be conducted as a hazard assessment. If conducted as a safety study, induction of sensitization should be a rare occurrence. Thus, follow-up studies are not meaningful since the number of sensitized volunteers would be low. However, following a series of the RIPTs with various concentrations of hydroxycitronellal, RIFM identified a group of 41 individuals who became sensitized. An extensive 3-phase use study, with 3 diagnostic patch tests and 4 whole-body dermatological examinations showed that most subjects were able to use a bar soap, a moisurizing lotion and cologne-type products with up to 1% hydroxycitronellal. In subjects where sensitization was induced by predictive testing, no serious recurring adverse dermatological conditions developed.

Begum, A; Katsumata, H; Kaneco, S; et al. (2003) **Microbial metabolism of phthalic acid esters by bakers' yeast Saccharomyces cerevisiae.** Fresenius Environ Bull 12:1309- 1314. The microbial metabolism behavior of two phthalic acid esters (PAEs), namely, diethyl phthalate (DEP) and butylbenzyl phthalate (BBP), from aqueous solution by bakers' yeast Saccharomyces cerevisiae was quantitatively characterized by high performance liquid chromatography (HPLC). The optimal culture temperature, amount of yeast, yeast growth time, and ammonium dihydrogen phosphate (ADP) amount, that can influence on biodegradation of PAEs were 30 degree C, 2.0 mg mL super(-1), 30 min, and 0.5 mg mL super(-1), respectively. Under the optimal conditions, the degradation percentage of DEP and BBP were 19% and 43%, respectively after 24 h of cultivation. The metabolites of DEP degradation as identified by gas chromatography-mass spectrometry (GC/MS) appeared to be ethyl methyl phthalate (EMP), dimethyl phthalate (DMP) and phthalic acid (PA), whereas monobutyl phthalate (MBP), diethyl phthalate (DEP) and phthalic acid (PA) were identified as metabolites of BBP. Therefore, it can be considered that one of the degradation pathways involves sequential cleavage of the ester bond to yield PA.

Buehler, EV. (1996) **Nonspecific hypersensitivity: false-positive responses with the use of Freund's complete adjuvant.** Contact Dermatitis 34:111-114. While conducting a guinea pig sensitization protocol, using the maximization test, it was discovered, at challenge, that the test animals were more responsive to the vehicle (acetone) than they were to the proprietary test material. During rechallenge, conducted to clarify the specific immune status of the test animals, it was determined that they were also hyperreactive to an alternate vehicle (diethyl phthalate), to which they were naive. This bizarre set of data is presented and it is suggested that this type of response is the prototype for the presence of false-positive responses experienced by toxicologists using this test. The test conditions imposed on the immune system by the maximization test that could result in these anomalous results are discussed. These data suggest that investigators need cautiously to interpret data that are produced by the injection of Freund's complete adjuvant (FCA).

CIVO INSTITUTE INC. (10/7) **PRIMARY SKIN AND EYE IRRITATION TESTS WITH A PERFUME CONCENTRATE (FV 8634) 10% IN DIETHYL PHTHALATE AND WITH DIETHYL PHTHALATE 100%, W/COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000891S OTS0557301 PRIMARY DERMAL IRRITATION, RABBITS, DERMAL; PRIMARY DERMAL IRRITATION, RABBITS, DERMAL; PRIMARY EYE IRRITATION, RABBITS, DERMAL; PRIMARY EYE IRRITATION, RABBITS, DERMAL

CIVO INSTITUTE INC. (12/7) **PRIMARY SKIN AND EYE IRRITATION TESTS OF ALPHA-ETHYLIDENE UNDECANAL 10% IN DIETHYL PHTHALATE AND 100% DIETHYL PHTHALATE WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000892S OTS0557302 PRIMARY DERMAL IRRITATION, RABBITS, DERMAL; PRIMARY DERMAL IRRITATION, RABBITS, DERMAL; PRIMARY EYE IRRITATION, RABBITS, DERMAL; PRIMARY EYE IRRITATION, RABBITS, DERMAL

CONSUMER PROD TESTING CO INC. (4/78) **GUINEA PIG SENSITIZATION (BUEHLER) TEST OF DIETHYL PHTHALATE (NEAT) WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000895S OTS0557305 PRIMARY DERMAL SENSITIZATION, GUINEA PIGS, DERMAL

Dear, WP; Jassup, DC. (1978) **Primary eye irritation study in the albino rabbit.** International Research and Development Corp.. tscat 878221260

Dolovich, J; Evans, S; Baurmeister, U; et al. (1987) **Antibody responses to hemodialysis-related antigens in chronic hemodialysis patients.** Artif Organs 11:93-96. Allergic-type reactions during hemodialysis are sometimes due to sensitization to ethylene oxide. To examine the possibility that additional antigens might be a basis for unexplained reactions, antibodies to formaldehyde and phthalate-related antigens and to dialyzer extracts were measured. Unselected sera from 113 chronic hemodialysis patients (CHP) and 200 control subjects were tested for IgG antibodies to formaldehyde-treated human serum albumin (HSA). The IgG antibody activity was confirmed in sera of five CHP who had used formaldehyde-treated dialyzers. Sera from 71 CHP and 80 controls were tested for IgE antibodies to diethylphthalate-treated HSA; antibody was detected in two CHP sera.

DOW CHEM CO. (8/52) **RESULTS OF SKIN IRRITATION TESTS ON DIETHYL PHTHALATE WITH COVER LETTER DATED 05/10/94 (SANITIZED).** 86940000816S OTS0557226 PRIMARY DERMAL IRRITATION, MAMMALS, DERMAL

ESSEX TESTING CLINIC INC. (11/8) **FINAL REPORT, CLINICAL SAFETY EVALUATION; REPEATED INSULT PATCH TEST OF DIETHYL PHTHALATE ALONE (100%) WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000900S OTS0557310 PRIMARY DERMAL SENSITIZATION, HUMANS, DERMAL

ESSEX TESTING CLINIC INC. (12/9) **FINAL REPORT, CLINICAL SAFETY EVALUATION OF TEST ARTICLE DIETHYL PHTHALATE CONTROL (NEAT); REPEATED INSULT PATCH TEST, WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000901S OTS0557311 PRIMARY DERMAL SENSITIZATION, HUMANS, DERMAL

ESSEX TESTING CLINIC INC. (5/89) **CLINICAL SAFETY EVALUATION OF DIETHYL PHTHALATE IN REPEATED INSULT PATCH TEST, WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000899S OTS0557309 PRIMARY DERMAL SENSITIZATION, HUMANS, DERMAL

Frosch, PJ; Pilz, B; Andersen, KE; et al. (1995) **Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes.** Contact Dermatitis 33:333-342. The objective of this study was to determine the frequency of reactivity to a series of commonly used fragrances in dermatological patients. A total of 48 fragrances (FF) were chosen, based on the publication of Fenn in 1989 in which the top 25 constituents of 3 types (1. perfumes, 2. household products, 3. soaps) of 400 commercial products on the US market had been determined. In a pilot study on a total of 1069 patients in 11 centres, the appropriate test concentration and vehicle were examined. For most fragrances, 1% and 5% were chosen, and petrolatum proved to be the best vehicle in comparison to isopropyl myristate and diethyl phthalate. In the main study, a set of 5 to 10 fragrances at 2 concentrations was patch tested in each centre on a minimum of 100 consecutive patients seen in the patch test clinic. These patients were also patch tested to a standard series with the 8% fragrance mix (FM) and its 8 constituents. In patients with a positive reaction to any of the 48 FF, a careful history with regard to past or present reactions to perfumed products was taken. A total of 1323 patients were tested in 11 centres. The 8% FM was positive in 89 patients (8.3% of 1072 patients). Allergic reactions to the constituents were most frequent to oak moss (24), isoeugenol (20), eugenol (13), cinnamic aldehyde (10) and geraniol (8). Reactions read as allergic on day 3/4 were observed only 10X to 7 materials of the new series (Iso E Super (2), Lyral (3), Cyclacet (1), DMBCA (1), Vertofix (1), citronellol (1) and amyl salicylate (1)). The remaining 41 fragrances were negative. 28 irritant or doubtful reactions on day 3/4 were observed to a total of 19 FF materials (more than 1 reaction: 5% citronellol (2), 1% amyl salicylate (2), 1% isononyl acetate (3), 0.1% musk xylol (2), 1% citral (2), and 1% ionone beta (2)). Clinical relevance of positive reactions to any of the FF series was not proved in a single case. This included the 4 reactions in patients who were negative to the 8% FM. In conclusion, the top 25 fragrances commonly found in various products caused few reactions in dermatological patients and these few appeared to be clinically irrelevant, with the possible exception of Lyral. However, this data should be interpreted in the light of the relatively small number of patients tested (only 100 in most centres).

HILL TOP RESEARCH INC. (1/68) **PRIMARY IRRITIATION PATCH TEST ON 3239K IN DIETHYL PHTHALATE AND ON DIETHYL PHTHALATE (NEAT) WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000896S OTS0557306 PRIMARY DERMAL IRRITATION, HUMANS, DERMAL; PRIMARY DERMAL IRRITATION, HUMANS, DERMAL

HILL TOP RESEARCH INST INC. (4/64) **REPEATED INSULT PATCH TEST OF DIETHYL PHTHALATE 12.5% IN ETHANOL WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000898S OTS0557308 PRIMARY DERMAL SENSITIZATION, HUMANS, DERMAL

Lalko, J; Isola, D; Api, AM. (2004) **Ethanol and diethyl phthalate: vehicle effects in the local lymph node assay.** Int J Toxicol 23:171-177. The vehicle in which an allergen is presented to the skin has been recognized to have an effect on the skin-sensitizing potency of the allergen. Typical vehicles used to evaluate the skin sensitization potential of fragrance materials include ethanol, diethyl phthalate, or a combination of the two. The authors conducted a series of studies to evaluate each of these vehicles for their utility in the murine local lymph node assay and to investigate the potential differences in skin sensitization resulting from their use. Four fragrance materials were tested in four different vehicles. The test materials were p-t-butyl- alpha-methylhydrocinnamic aldehyde, geraniol, eugenol, and hydroxycitronellal. The vehicles were diethyl phthalate, 1:3 ethanol:diethyl phthalate, 3:1 ethanol:diethyl phthalate, and ethanol. Each of the fragrance materials was tested at five dose levels ranging from 0.3% to 50% w/v. In all four vehicles, each material tested elicited positive responses, exhibiting weak to moderate skin sensitization potential. Overall, p-t- butyl-alpha-methylhydrocinnamic aldehyde exhibited the most potency, followed by eugenol, geraniol, and hydroxycitronellal. The sensitization potential of both p-t-butyl-alpha-methylhydrocinnamic aldehyde and geraniol was greatest when the vehicle was ethanol. The sensitization potential of eugenol was greatest in 3:1 ethanol:diethyl phthalate, but the sensitization potential for hydroxycitronellal was greatest in 1:3 ethanol:diethyl phthalate. The strength of the sensitization response was observed to vary with the vehicle; however, the results did not show any clear pattern of one vehicle over another regarding skin sensitization.

LEBERCO LABORATORIES. (2/78) **RABBIT SKIN IRRITATION TEST OF DIETHYL PHTHALATE WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000893S OTS0557303 PRIMARY DERMAL IRRITATION, RABBITS, DERMAL

Oliwiecki, S; Beck, MH; Chalmers, RJ. (1991) **Contact dermatitis from spectacle frames and hearing aid containing diethyl phthalate.** Contact Dermatitis 25:264-265.

Olsen, PR; Larsen, NA; Faurby, V; et al. (1982) **Nephrotoxicity of plasticizers investigated by 48 hours hypothermic perfusion of dog kidneys.** Scand J Urol Nephrol 16:187-190. The possible nephrotoxicity of the plasticizers diethyl phthalate and di-2-ethylhexyl phthalate was tested in vitro using a 48 hours continuous pulsatile hypothermic perfusion of canine kidneys with a human albumin perfusion medium. Since polysorbate 80 was used to facilitate the solution of the plasticizers in the perfusion medium, this substance was also tested. Six groups containing 9--15 kidneys were perfused with different amounts of plasticizers and/or polysorbate 80 added. In perfusates containing polysorbate 80 either alone or with one of the two plasticizers, the LDH activity and the potassium concentrations rose significantly higher than in the control group (p less than 0.001). The kidney weight gain was also significantly greater in these groups. A 'blind' histological examination of needle biopsies by light microscopy revealed no differences among the groups. Although the biochemical evidence of tissue damage was not tested by re-implantation of the kidneys, we suggest that caution should be exercised in the use of polysorbate 80 in organ perfusion systems.

PHARMAKON RESEARCH INTL INC. (9/82) **SINGLE RABBIT DERMAL PILOT STUDY OF DIETHYL PHTHALATE WITH COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000889S OTS0557299 PRIMARY DERMAL IRRITATION, RABBITS, DERMAL

Politano, VT; Isola, DA; Lalko, J; et al. (2006) **The effects of vehicles on the human dermal irritation potentials of allyl esters.** Int J Toxicol 25:183-193. Allyl esters, frequently used in the fragrance industry, often contain a certain percentage of free allyl alcohol. Allyl alcohol is known to have a potential for delayed skin irritation. Also present in the finished product are different solvent systems, or vehicles, which are used to deliver the fragrances based upon their intended application. This study was conducted to determine whether different vehicles affect the skin irritation potential of five different allyl esters. The allyl esters tested were allyl amyl glycolate, allyl caproate, allyl (cyclohexyloxy)acetate, allyl cyclohexylpropionate, and allyl phenoxyacetate in the vehicles diethyl phthalate, 3:1 diethyl phthalate:ethanol, and 1:3 diethyl phthalate:ethanol at concentrations of 0.1%, 0.5%, 1.0%, and 2.0% (w/w). A modified cumulative irritation test was conducted in 129 human subjects. Test materials (0.3 ml) were applied under occlusion to skin sites on the back for 1 day (24 h) using Hill Top chambers. Irritation was assessed at

1, 2, 4, and 5 days following application of test materials. Cumulative irritation scores varied considerably among test materials. There were no delayed irritation observations. The highest irritation scores were observed at the 2.0% concentration for all test materials. The irritation scores for allyl amyl glycolate, allyl (cyclohexyloxy)acetate, and allyl phenoxyacetate were highest in 1:3 diethyl phthalate:ethanol, thus the resulting calculated no-observed-effect levels, 0.12%, 0.03%, and 0%, respectively, were much lower for this vehicle compared to the diethyl phthalate vehicle, 0.33%, 0.26%, 0.25%, respectively. These data showed a trend for lower concentration thresholds to induce irritation when higher levels of ethanol were used in the vehicle.

Schulsinger, C; Mollgaard, K. (1980) **Polyvinyl chloride dermatitis not caused by phthalates.** Contact Dermatitis 6:477-480. Seven cases of contact dermatitis in children due to identification bracelets made of polyvinyl chloride plastic are reported. Patch tests with the bracelets were negative in the five cases tested. It is concluded that the reactions were irritant due to some unknown chemical in the bracelets. The most widely used plasticizers in PVC, phthalates, must have very low sensitizing properties, as only one positive patch test was found in 1532 patch tests with phthalate mix, performed as a joint study by the International Contact Dermatitis Research Group.

TECHNI-MED CONSULTANTS INC. (3/85) **EVALUATION TO DETERMINE HAZARDS OF SKIN IRRITATION BY DERMAL CONTACT WITH PI-3745 AND PI-3746 IN DILUENTS PI-3747 AND DIETHYL PHTHALATE, W/COVER LETTER DATED 05/09/94 (SANITIZED).** 86940000897S OTS0557307 PRIMARY DERMAL IRRITATION, HUMANS, DERMAL; PRIMARY DERMAL IRRITATION, HUMANS, DERMAL

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

Agarwal, DK; Lawrence, WH; Nunez, LJ; et al. (1985) **Mutagenicity evaluation of phthalic acid esters and metabolites in Salmonella typhimurium cultures.** J Toxicol Environ Health 16:61-69. The mutagenic potential of dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-2-ethylhexyl phthalate (DEPH), as well as metabolites of DEHP--i.e., mono-2-ethylhexyl phthalate (MEHP), 2-ethylhexanol (2-EH), and phthalic acid (PA)--were tested in Salmonella typhimurium cultures using the Ames test procedure. The compounds were tested on strains TA98, TA100, TA1535, TA1537, TA1538, and TA2637 for base-pair substitution or frameshift-type mutations. Spot tests yielded negative responses for all compounds with the strains tested. Each compound was tested for a dose-effect relationship in the TA98, TA100, TA1535, and TA1538 systems. DEP and DBP exhibited a mildly positive response in both TA100 and TA1535 cultures, and DMP showed a similar response in TA1535. Normalization of the data for cytotoxicity of DMP suggests TA100 has a mildly positive effect. The higher doses of these compounds exhibited some cytotoxic effects. The mutagenic effects were apparently abolished by the addition of S9 fraction in TA100 and TA1535 cultures, while no effect, other than cytotoxicity, was observed in the TA98 and TA1538 systems. DEHP, MEHP, 2-EH, and PA exhibited no mutagenicity in any of the strains of Salmonella typhimurium tested, with or without S9 metabolic activation. MEHP and 2-EH, however, exhibited a moderate cytotoxic effect in most cultures.

Ashour, MB; Moody, DE; Hammock, BD. (1987) **Apparent induction of microsomal carboxylesterase activities in tissues of clofibrate-fed mice and rats.** Toxicol Appl Pharmacol 89:361-369. Treatment with 0.5% (w/w) dietary clofibrate, a peroxisome proliferator, for 14 days induced microsomal carboxylesterase activities for five substrates including malathion, clofibrate, diethylsuccinate, diethylphthalate, and p-nitrophenylacetate in liver and kidney of male Swiss-Webster mice and Sprague-Dawley rats. The induction was substrate, tissue, and species dependent. The carboxylesterase activity was induced in mouse from 1.2- to 2.2-fold (liver) and from 1.1- to 1.7-fold (kidney) depending upon substrate used. Analogous values from rat ranged from 1.0- to 1.4-fold (liver) and from 1.1- to 1.8-fold (kidney). Enzyme activities were either decreased or not affected in testes of treated mice and rats. Substituted trifluoroketones ('transition-state' inhibitors of carboxylesterase) were found to be very potent inhibitors of clofibrate-metabolizing carboxylesterase(s) and to be potentially useful in distinguishing among isozymes. The inhibition data suggested that changes in carboxylesterase activity following clofibrate treatment were both qualitative and quantitative.

BASF CORP. (5/94) **LETTER FROM BASF CORP TO USEPA REGARDING SUBMISSION OF HEALTH AND SAFETY DATA SUBJECT TO 8D REPORTING WITH ATTACHMENTS, DATED 05/05/94 (SANITIZED) NOTE: STUDIES ARE IN GERMAN.** 86940001380S OTS0572606 GENE MUTATIONS, BACTERIA, IN VITRO

BASF CORP. (6/93) **LETTER FROM BASF CORP TO USEPA RE: REPORT ON THE STUDY OF PALATINOL A (DEP) (ZST TEST SUBSTANCE NO. 92/187) IN THE AMES TEST W/ATTACHMENTS & COVER LETTER DATED 05/06/94 (SANITIZED).** 86940000390S OTS0572493 GENE MUTATIONS, BACTERIA, IN VITRO

Blevins, RD; Taylor, DE. (1982) **Mutagenicity screening of twenty-five cosmetic ingredients with the Salmonella/microsome test.** J Environ Sci Health Part A 17:217-239.

DeMarini, DM; Inmon, JP; Simmons, JE. (1987) **Mutagenicity in Salmonella of hazardous wastes and urine from rats fed these wastes.** Mutat Res 189:205-216.

DOW CORNING CORP. (6/87) **GENETIC EVALUATION OF MOLYKOTE PENE-LUBE, DIETHYL PHTHALATE, IN BACTERIAL REVERSE MUTATION ASSAYS WITH COVER LETTER DATED 031194.** 86940000162 OTS0556757 GENE MUTATIONS, BACTERIA, IN VITRO

Gollamudi, R; Lawrence, WH; Rao, RH; et al. (1985) **Effects of phthalic acid esters on drug metabolizing enzymes of rat liver.** J Appl Toxicol 5:368-371. Di(2-ethylhexyl)phthalate (DEHP) inhibited UDP-glucuronyltransferase activity of rat liver in vitro and in vivo. Diethyl phthalate and dimethoxyethyl phthalate also inhibited this enzyme in vitro. On the other hand, DEHP did not inhibit the activity of the cytosolic enzyme N-acetyltransferase; it also did not alter the levels of rat liver microsomal cytochrome P-450 in vitro. It is suggested that DEHP may alter the composition of microsomal phospholipids.

Harris, CA; Henttu, P; Parker, MG; et al. (1997) **The estrogenic activity of phthalate esters in vitro.** Environ Health Perspect 105:802-811. A large number of phthalate esters were screened for estrogenic activity using a recombinant yeast screen. a selection of these was also tested for mitogenic effect on estrogen- responsive human breast cancer cells. A small number of the commercially available phthalates tested showed extremely weak estrogenic activity. The relative potencies of these descended in the order butyl benzyl phthalate (BBP) > dibutyl phthalate (DBP) > diisobutyl phthalate (DIBP) > diethyl phthalate (DEP) > diisononyl phthalate (DINP). Potencies ranged from approximately 1 x 10(6) to 5 x 10(7) times less than 17beta-estradiol. The phthalates that were estrogenic in the yeast screen were also mitogenic on the human breast cancer cells. Di(2-ethylhexyl) phthalate (DEHP) showed no estrogenic activity in these in vitro assays. A number of metabolites were tested, including mono-butyl phthalate, mono-benzyl phthalate, mono-ethylhexyl phthalate, mon-n-octyl phthalate; all were wound to be inactive. One of the phthalates, ditridecyl phthalate (DTDP), produced inconsistent results; one sample was weakly estrogenic, whereas another, obtained from a different source, was inactive. analysis by gel chromatography-mass spectometry showed that the preparation exhibiting estrogenic activity contained 0.5% of the ortho-isomer of bisphenol A. It is likely that the presence of this antioxidant in the phthalate standard was responsible for the generation of a dose-response curve--which was not observed with an alternative sample that had not been supplemented with o,p'-bisphenol A--in the yeast screen; hence, DTDP is probably not weakly estrogenic. The activities of simple mixtures of BBP, DBP, and 17beta-estradiol were assessed in the yeast screen. No synergism was observed, although the activities of the mixtures were approximately additive. In summary, a small number of phthalates are weakly estrogenic in vitro. No data has yet been published on whether these are also estrogenic in vitro. No data has yet been published on whether these are also estrogenic in vivo; this will require tests using different classes of vertebrates and different routes of exposure.

Hong, EJ; Ji, YK; Choi, KC; et al. (2005) **Conflict of estrogenic activity by various phthalates between in vitro and in vivo models related to the expression of Calbindin-D9k.** J Reprod Dev 51:253-263. Phthalates are suspected to disrupt the endocrine system, especially through estrogenic effects. In the present study, we investigated the effects of various phthalates and compared them with those of estrogenic compounds that disrupt the female reproductive system. To assess the effects of these phthalates, alteration of the Calbindin-D9k (CaBP-9k)

gene was measured as a biomarker because rat CaBP-9k gene carries an estrogen response element (ERE) which is involved in estrogen responsiveness of the gene during the estrous cycle. In this study, phthalates were tested for estrogenic properties in in vitro and in vivo models. First, the E-Screen assay was used to measure the proliferation of MCF-7 cells, a human breast cancer cell line. Treatments with 17beta-estradiol (E2; 9-fold) and 17alpha-estradiol (EE; 9-fold) induced MCF-7 cell proliferation at concentrations of 10(-9) M. Phthalates induced an increase in MCF-7 proliferation at concentration of 10(-6) M up to 10(-4) M. Nbutyl benzyl phthalate (BBP; 6-fold vs. vehicle), dicyclohexyl phthalate (DCHP; 8-fold), 2-ethylhexyl phthalate (DEHP; 6-fold) and di-n-butyl phthalate (DBP; 7-fold) at the concentration of 10(-4) M induced in an increase in MCF-7 proliferation after 6 d of treatment compared to vehicle. However, significant increase in MCF-7 proliferation was induced by diethyl phthalate (DEP). Second, we investigated the expression of CaBP-9k in the uterus of immature rats after oral treatment with BBP, DCHP, DEHP, DBP or DBP (600 mg/kg per day) in this in vivo model, because the immature rat model is highly sensitive to exposure to estrogenic chemicals. None of the phthalates induced the expression of CaBP-9k mRNA and its protein in the neonatal uterus as analysed by Northern and Western blot analyses, respectively. Although phthalates induced an increase in MCF-7 cell proliferation by an estrogenic effect, they could not induce CaBP-9k expression in the in vivo system, suggesting that the assays of estrogenic effects of various phthalates conducted in vitro and in vivo expression of CaBP-9k may produce conflicting results.

Hwang, DY; Cho, JS; Oh, JH; et al. (2005) **An in vivo bioassay for detecting antiandrogens using humanized transgenic mice coexpressing the tetracycline-controlled transactivator and human CYP1B1 gene.** Int J Toxicol 24:157-164. The typical strategy used in analysis of antiandrogens involves the morphological changes of a marker in castrated rats Hershberger assay for the prostate, seminal vesicle, levator ani plus bulbocavernosus muscles (LABC), Cowper's gland, and glans penis. However, there are disadvantages to this approach, such as the time required, and the results may not correspond to those in actual human exposure. To evaluate its ability for detecting antiandrogens, in vivo the dose effect of di-(2-ethylhexyl) phthalate (DEHP) and time effect of five antiandrogens, DEHP, di-n-butyl phthalate (DBP), diethyl phthalate (DEP), linuron (3-(4-dichlorophenyl)-methoxy-1-methylurea), and 2,4'-DDE (1,1-dichloro-2-(p- chlorophenyl)-2-(o-chlorophenyl)ethylene), were investigated using humanized transgenic mice coexpressing tetracycline-controlled transactivator (tTA) and the human cytochrome P450 (CYP) enzyme CYP1B1 (hCYP1B1). Adult transgenic males were treated with each of the five antiandrogens, and their tTA-driven hCYP1B1 expressions analyzed by real-time polymerase chain reaction (PCR) and/or Western blot and for O-debenzylation activity. Herein, the treatments of adult males with the five antiandrogens were shown to affect the increased levels of tTA-driven hCYP1B1 expression in both dose-dependent and repeated experiments. Thus, this novel in vivo bioassay, using humanized transgenic mice, is useful for measuring antiandrogens, and is a means to a more relevant bioassay relating to actual human exposure.

Igarashi, A; Ohtsu, S; Muroi, M; et al. (2006) **Effects of possible endocrine disrupting chemicals on bacterial component-induced activation of NF-kappaB.** Biol Pharm Bull 29:2120-2122. Endocrine disrupting chemicals (EDCs) have a possibility to exacerbate infectious diseases because EDCs disturb the human immune system by interfering with endocrine balance. To assess the influence of EDCs on the innate immune function of macrophages, we investigated the effects of thirty-seven possible endocrine disruptors on lipopolysaccharide (LPS)- or bacterial lipopeptide (Pam3CSK4)-induced activation of nuclear factor kappa B (NF-kappaB). Alachlor, benomyl, bisphenol A, carbaryl, kelthane, kepone, octachlorostyrene, pentachlorophenol, nonyl phenol, p-octylphenol and ziram inhibited both LPS- and Pam3CSK4-induced activation of NF-kappaB. Simazine inhibited only LPS-induced activation. A strong inhibitory effect was observed with ziram and benomyl. On the other hand, diethylhexyl adipate and 4-nitrotoluene tended to enhance the activation induced by Pam3CSK4 and LPS, respectively. Aldicarb, amitrole, atrazine, benzophenone, butyl benzyl phthalate, 2,4-dichlorophenoxy acetic acid, dibutyl phthalate, 2,4-dichlorophenol, dicyclohexyl phthalate, diethylhexyl phthalate, diethyl phthalate, dihexyl phthalate, di-n- pentyl phthalate, dipropyl phthalate, malathion, methomyl, methoxychlor, metribuzin, nitrofen, permethrin, trifluralin, 2,4,5-trichlorophenoxyacetic acid and vinclozolin had no significant effects at 100 microM. These results indicate that some agrochemicals have the potential to inhibit macrophage function and suggest that endocrine disruptors may influence the development of bacterial infections.

Ishidate, M; Odashima, S. (1977) **Chromosome tests with 134 compounds on Chinese hamster cells in vitro - A screening test for chemical carcinogens.** Mutat Res 48:337-354. Chromosomal aberration tests in vitro were carried out on Chinese hamster cells grown in culture with various chemicals, including carcinogenic N-nitroso compounds and their related derivatives, food additives, medical drugs, pesticides and other chemicals commonly

used in laboratories or industries. Sixty-three of the 134 chemicals gave negative results in our test system even with doses at which the cell growth was markedly inhibited. Nearly all compounds known to be mutagenic in bacteria were also positive in our tests. Both urethane and diethylstilbestrol were positive, even though they are known to be carcinogenic but not mutagenic in bacteria. Compounds such as N-alkyl-N'-nitroguanidines, barbital, sodium benzoate, saccharin sodium, sodium nitrite, sodium nitrate and 4-aminoquinoline-1-oxide were positive in our chromosome tests, but they have not been conclusively tested for their carcinogenicity.

Kozumbo, WJ; Kroll, R; Rubin, RJ. (1982) **Assessment of the mutagenicity of phthalate esters.** Environ Health Perspect 45:103-109. The Ames assay was used to investigate the mutagenicity of several phthalate esters as an approximation of their carcinogenic potential. The ortho diesters, dimethyl phthalate (DMP) and diethyl phthalate (DEP) produced a positive dose-related mutagenic response with Salmonella TA100, but only in the absence of S-9 liver enzymes. Dibutyl, di(2-ethylhexyl), mono(2- ethylhexyl), and butyl benzyl phthalate as well as the dimethyl isophthalate and terephthalates and the trimethyl ester, trimellitate, were not mutagenic with TA100 or TA98 in the presence or absence of S-9. In a host-mediated assay, extracts of 24-hr urines of rats injected IP with DMP (2 g/kg) were not mutagenic to TA100 at levels up to 8 equivalent-ml of urine/plate (representing 30% of their daily urinary output). In vitro studies revealed that S-9 associated esterase hydrolyzed DMP to the monoester and methanol and eliminated its mutagenicity. Whole rat skin was shown to have about 1.5% of the DMP-esterase activity of liver, when compared on a wet weight basis. An in vitro binding study indicated that epidermal macromolecules bound DMP at a severalfold greater rate than hepatic macromolecules. Thus, both the mutagenicity and binding of DMP are inversely related to the metabolism of this compound. These results suggest that skin could be at high risk for a mutagenic/carcinogenic insult.

Liu, K; Lehmann, KP; Sar, M; et al. (2005) **Gene expression profiling following in utero exposure to phthalate esters reveals new gene targets in the etiology of testicular dysgenesis.** Biol Reprod 73:180-192. Male reproductive tract abnormalities associated with testicular dysgenesis in humans also occur in male rats exposed gestationally to some phthalate esters. We examined global gene expression in the fetal testis of the rat following in utero exposure to a panel of phthalate esters. Pregnant Sprague-Dawley rats were treated by gavage daily from Gestational Days 12 through 19 with corn oil vehicle (1 ml/kg) or diethyl phthalate (DEP), dimethyl phthalate (DMP), dioctyl tere-phthalate (DOTP), dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP), dipentyl phthalate (DPP), or benzyl butyl phthalate (BBP) at 500 mg/kg per day. Testes were isolated on Gestational Day 19, and global changes in gene expression were determined. Of the approximately 30 000 genes queried, expression of 391 genes was significantly altered following exposure to the developmentally toxic phthalates (DBP, BBP, DPP, and DEHP) relative to the control. The developmentally toxic phthalates were indistinguishable in their effects on global gene expression. No significant changes in gene expression were detected in the nondevelopmentally toxic phthalate group (DMP, DEP, and DOTP). Gene pathways disrupted include those previously identified as targets for DBP, including cholesterol transport and steroidogenesis, as well as newly identified pathways involved in intracellular lipid and cholesterol homeostasis, insulin signaling, transcriptional regulation, and oxidative stress. Additional gene targets include alpha inhibin, which is essential for normal Sertoli cell development, and genes involved with communication between Sertoli cells and gonocytes. The common targeting of these genes by a select group of phthalates indicates a role for their associated molecular pathways in testicular development and offers new insight into the molecular mechanisms of testicular dysgenesis. **(Also 4.6)**

Lu KaunYi; Tseng FuWei; Wu ChaiJung; et al. (2004) **Suppression by phthalates of the calcium signaling of human nicotinic acetylcholine receptors in human neuroblastoma SH-SY5Y cells.** Toxicology 200:113-121. Phthalates are widely used in industry and cause public concern since they have genomic estrogenic-like effects via estrogen receptors. We previously found that some phthalates have nongenomic effects, exerting inhibitory effects on the functional activities of nicotinic acetylcholine receptors (nAChRs) in bovine chromaffin cells. In this study, we investigated the effects of eight phthalates on the calcium signaling of human nAChR by using human neuroblastoma SH-SY5Y cells. All eight phthalates, with different potency, have inhibitory roles on the calcium signaling coupled with human nAChR, but not muscarinic acetylcholine receptors (mAChRs). For inhibition of human nAChR, the strongest to weakest potencies were observed as di-n-pentyl phthalate (DPP) --> butyl benzyl phthalate (BBP) --> di-n-butyl phthalate (DBP) --> dicyclohexyl phthalate (DCHP) --> di-n-hexyl phthalate (DHP) --> di- (2-ethyl hexyl) phthalate (DEHP) --> di-n-propyl phthalate (DPrP) --> diethyl phthalate (DEP). The potencies of phthalates were associated with their structures such that the most effective ones had dialkyl group carbon numbers of C4 or C5, with shorter or longer numbers resulting in decreased potency. At as low as 0.1 microM, DPP,

DBP, BBP, DCHP and DHP significantly inhibited the calcium signaling of human nAChR. The IC50 of phthalates on human nAChR, ranging from 0.32 to 7.96 microM, were 10-50 lower than those for bovine nAChR. We suggest that some phthalates effectively inhibit the calcium signaling of human nAChR, and these nongenomic effects are cause for concern.

Okita, RT; Okita, JR. (1992) **Effects of diethyl phthalate and other plasticizers on laurate hydroxylation in rat liver microsomes.** Pharm Res 9:1648- 1653. Diethyl phthalate (DEP) is used in pharmaceutical coatings, cosmetics, and plastic films to wrap foods. There is a health concern associated with the exposure to certain phthalate esters because they belong to a class of compounds referred to as peroxisome proliferators which have been shown to increase the incidence of liver tumors when administered to rats. In this study, we have compared DEP to four other commonly used plasticizers, 2-diethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), 2-diethylhezyl adipate (DEHA), and acetyltributyl citrate (ATBC), for their ability to induce the cytochrome P450-mediated fatty acid omega-hydroxylation system, which is one of the initial cellular responses when animals are treated with peroxisome proliferators. The administration of DEHP, DBP, and DEHA to rats increased the specific activity of laurate 12-hydroxylase from 2.8 +/- 1.1 in control rats to 30.3 +/- 11.6, 14.5 +/- 4.1, and 9.7 +/- 1.9 nmol 12-hydroxylaurate formed/min/nmol P450, respectively. In contrast, laurate 12-hydroxylase activity in DEP- and ATBC-treated rats were 4.4 +/- 1.2 and 4.4 +/- 1.0 nmol 12-hydroxylaurate formed/min/nmol P450, respectively. In addition, whereas DEHP increased peroxisomal palmitoyl-CoA oxidation 6-fold, DEP increased this activity only 1.3-fold. Two protein bands, at 51 and 52 kDa, were found to increase 6- to 12-fold in microsomes of DEHP-, DBP-, and DEHA- treated rats, but these bands were increased only 2-fold in DEP- or ATBC-treated rats.

Seed, JL. (1982) **Mutagenic activity of phthalate esters in bacterial liquid suspension assays.** Environ Health Perspect 45:111-114. The mutagenic activities of several phthalate esters have been evaluated in an 8- azaguanine resistance assay in Salmonella typhimurium. Three phthalate esters were found to be mutagenic: dimethyl phthalate, diethyl phthalate and di-n-butyl phthalate. A number of other phthalate esters were not found to be mutagenic, including di(2-ethylhexyl) phthalate, di-n-octyl phthalate, diallyl phthalate, diisobutyl phthalate and diisodecyl phthalate. A metabolite of di(2-ethylhexyl) phthalate, 2- ethylhexanol, was also noted to be mutagenic. The mutagenic activity of this agent and others in this series was dose dependent but weak. No dose-response curve exceeded more than 3.5 times background at maximally testable concentrations. A liquid suspension histidine reversion assay of dimethyl phthalate showed levels of mutagenic activity similar to that observed in the azaguanine resistance assay. The data suggest a need for further investigation of the mutagenic potential of these agents in other assay systems.

Tsuchiya, K; Hattori, K. (1977) **Chromosomal study on human leukocyte cultures treated with phthalic acid ester.** Hokkaidoritus Eisei Kenkyusho Ho 26:114.

Zeiger, E; Haworth, E; Mortelmans, S. (1985) **Mutagenicity testing of di(2-ethylhexyl) phthalate and related chemicals in Salmonella.** Environ Mutagen 7:213-232. Di(2-ethylhexyl)phthalate and 33 other phthalates, ethylhexanol derivatives, and related chemicals were tested for mutagenicity in Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 without metabolic activation and in the presence of rat and hamster liver S-9 metabolic activation systems. No mutagenic activity was seen with any of the chemicals tested.

Zeiger, E; Haworth, E; Speck, S. (1982) **Phthalate ester testing in the National Toxicology Program's environmental mutagenesis test development program.** Environ Health Perspect 45:99-101. A number of phthalate esters and related chemicals were tested for mutagenicity in Salmonella typhimurium. The chemicals were tested blind in three laboratories by a preincubation modification of the Ames Salmonella/mammalian microsome test using S-9 prepared from Aroclor-induced rats and Syrian hamsters. All chemicals tested were judged to be nonmutagenic.