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**Committee of Experts on the Transport of Dangerous Goods and on the Globally Harmonized System of Classification and Labelling of Chemicals**

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Item 4 (a) of the provisional agenda
**Implementation of the GHS:**
**Development of a list of chemicals classified in accordance with the GHS**

## Report on the proposal for classification and labelling of Dibutyl Phthalate: Annex 1

**Transmitted by the secretariat of the Organisation for Economic Cooperation and Development (OECD)**

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

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English - Or. English

**ENVIRONMENT DIRECTORATE**
**JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

# ANNEX 1 TO : REPORT ON THE PROPOSAL FOR CLASSIFICATION AND LABELLING (C&L) OF DIBUTYL PHTHALATE

**Series on Testing & Assessment**
**No. 249**

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## Series on Testing & Assessment

## No. 249

**ANNEX 1 TO:**

**REPORT ON THE PROPOSAL FOR CLASSIFICATION AND LABELLING (C&L) OF DIBUTYL PHTHALATE**

**Joint Pilot Project of the OECD and the UN Sub-Committee of Experts on the Globally Harmonised System of Classification and Labelling of Chemicals**

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## FOREWARD

This document is Annex 1 to the Report on the Proposal for Classification and Labelling (C&L) of Dibutyl Phthalate.

This document is published under the responsibility of the Joint Meeintg of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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## APPENDIX 1: BACKGROUND INFORMATION

Summary tables of information selected for several toxicology endpoints were too lengthy to include in the main body of the classification document, making that document difficult to read. Therefore the tables are presented in this appendix. Final summaries derived from these tables are in the respective sections of the classification document.

Pease note that, in many cases, abstracts of publications were used as the basis for these tables and the original cited publications were not reviewed due to constraints in time. Therefore determinations of the quality of the publications were generally not performed. However, several of the publications have been previously cited in earlier reviews in which such evaluations were performed.

## BACKGROUND INFORMATION FOR TABLE 25: TOXICOKINETIC STUDIES

| Summary of Study | Reference |
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| **Type/Objective:** To dertermine metabolites of DBP in rat urine<br>**Conclusion:** Di-n-butyl-phthalate is metabolized by hydrolysis of one ester bond and oxidation of the remaining alkyl chain.<br>**Method:** Adult male CD-rats received 0.2 mL doses of DBP by gavage at 24-hr intervals. Urine was collected for 24 hr following the initial dose. Individual metabolites or individual homologous series of metabolites were isolated by pressure assisted liquid chromatography.<br>**Results:** Six metabolites were detected in the urine of rats fed di-n-butyl-phthalate. A trace of intact di-n-butyl-phthalate was found including a 7 membered homologous series. | Albro and Moore, 1974 |
| **Type/Objective:** To investigate role of intestinal esterases in absorption on phthalate diesters in everted rat gut sacs<br>**Conclusion:** The authors suggest that intestinal esterases are important in the absorption and metabolism of phthalate diesters.<br>**Method:** The absorption and metabolism of mono-n-**butyl-phthalate** (34742) (MBP), di-n-**butyl-phthalate** (DBP), monomethyl-phthalate (MMP), dimethyl-phthalate (131113) (DMP), di-(2-ethylhexyl)-phthalate (117817) (DEHP), mono-(2-ethyl-hexyl)-phthalate (MEHP) and (carbon-14) carbonyl-phthalate esters by the small intestine were studied in everted gut sac preparations from rats.<br>**Results:** All phthalate monoesters were absorbed in a larger quantity than their corresponding diesters. Esterases hydrolyzed the phthalate diesters during absorption; 81.2 percent of the dimethyl, 95.5 percent of the di-n-butyl and 100 percent of the di(2-ethyl-hexyl) esters were hydrolyzed to the monoester. Absorption of di-n-**butyl-phthalate** was reduced when the esterose activity of the mucosa was decreased by intragastric treatment with an organophosphate. Esterase inhibition did not affect the absorption of mono-n-**butyl-phthalate**. | White et al, 1980 |
| **Type/Objective:** To measure dermal absorption of DBP in rat skin<br>**Conclusion:** DBP is dermally absorbed.<br>**Method:** A single dose of 157 μmol/kg 14C-DBP was applied to the shaved backs of male F344 rats and the site was covered with a perforated cap.<br>**Results:** Dermal absorption resulted in a constant rate of urinary excretion of 10-12% of dose in 24 hours for the 7 days urine samples were collected. In addition, at the end of the 7 days between 0.41 and 1.4% of the dose was collected from other tissues. | Elsisi et al, 1989, as summarized in CPSC, 2010 |
| **Type/Objective:** To test PBPK models for a less lipophilic phthalate diester, di(n-**butyl) phthalate** (DBP), and monoester, mono(n-**butyl) phthalate** (MBP).<br>**Conclusion:** The application of the pH trapping model is a step toward developing a generic model structure for all phthalate esters, though more work is required before a generic structure can be identified with confidence.<br>**Method:** Alternate models describing enterohepatic circulation, diffusion- | Keys et al, 2000 |

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| limitation, tissue pH gradients (pH trapping), and a simpler, flow-limited model were evaluated. A combined diffusion-limited and pH trapping model was also tested. MBP tissue:blood partition coefficients were similar when determined either experimentally by a nonvolatile, vial equilibration technique or algorithmically. All other parameters were obtained from the literature or estimated from MBP blood concentrations following intravenous or oral exposure to DBP or MBP.<br>**Results:** A flow-limited model was unable to predict MBP blood levels, whereas each alternative model had statistically better predictions. The combined diffusion-limited and pH trapping model was the best overall, having the highest log-likelihood function value. This result is consistent with a previous finding that the pH trapping model was the best model for describing DEHP and MEHP blood dosimetry, though it was necessary to extend the model to include diffusion-limitation. | |
| **Type/Objective:** To measure kinetics of oral DBP in rats<br>**Conclusion:** DBP is readily absorbed from the GI tract and 63 - ≥90% of the administered radioactivity was excreted in urine within 48h. Fecal excretion is low.<br>**Method:**<br>**Results:** Oral studies in rats and hamsters given 14C-DBP, showed that DBP is readily absorbed from the gastrointestinal tract; 63 - ≥90% of the administered radioactivity was excreted in urine within 48h (Foster et al., 1982; Tanaka et al., 1978; Williams and Blanchfield, 1975). Fecal excretion was low (1.0-8.2%) (Tanaka et al., 1978). | EC, 2003 (section 4.1.2.1.1) |
| **Type/Objective:** To measure DBP in blood of people after ingestion of food containing DBP<br>**Conclusion:** Oral absorption of DBP was indicated.<br>**Method:**<br>**Results:** In 13 individuals who had ingested food which had been in contact with plastic packaging material containing DBP, a mean blood level of 0.10 mg DBP/L was found, while the mean blood level of 9 unexposed men was 0.02 mg/L. These figures indicate oral absorption of DBP also by humans (Tomita et al., 1977). | EC, 2003 (section 4.1.2.1.1) |
| **Type/Objective:** To measure dermal absorption and excretion of DBP in rats<br>**Conclusion:** 60% of applied radioactive label was excreted in urine in 7 days. Fecal excretion was lower.<br>**Method:** 14C-DBP in ethanol was applied to the clipped skin (circular area with diameter of 1.3 cm) of male F344 rats (bw 180-220 g).<br>**Results:** 10-12% of the administered dose per day was excreted in urine for a total of ca. 60% within 7 days. In feces ca.1% of the dose was excreted in 24 hours (totally ca. 12% within 7 days) (Bronaugh et al., 1982; Elsisi et al., 1989). | EC, 2003 (section 4.1.2.1.1) |
| **Type/Objective:** Placental transfer study pregnant Sprague-Dawley rats<br>**Conclusion:** DBP and its metabolites (MBP and MBP-glucuronire were readily transferred to embryos.<br>**Method:**<br>**Results:** In a placental transfer study pregnant Sprague-Dawley rats received a single oral dose of 500 or 1,500 mg 14C-labelled DBP/kg bw on day 14 of gestation. Maternal and fetal tissues were collected at intervals from 0.5 to 48 hours. Radioactivity in embryonic tissues accounted for less than 0.12-0.15% of the administered dose. Levels of radioactivity in placenta and embryo were 1/3 or less of those in maternal plasma. No accumulation of radioactivity was observed in maternal or embryonic tissues. It was shown that unchanged DBP and its | EC, 2003 (section 4.1.2.1.1) |

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| metabolites MBP and MBP-glucuronide were rapidly transferred to the embryonic tissues, where their levels were constantly lower than those in maternal plasma. MBP accounted for most of the radioactivity recovered in maternal plasma, placenta and embryo. Unchanged DBP was found only in small amounts (Saillenfait et al., 1998). | |
| **Type/Objective:** To measure distribution of DBP in rats after one oral dose.<br>**Conclusion:** Rats did not show significant retention in any organ.<br>**Method:** Male Wistar rats which had received a single oral dose of 0.27 or 2.31 g 14C-DBP/kg bw in corn oil.<br>**Results:** Distribution was similar after both dose levels. The lowest amount of activity was found in the brain (0.03%) and the highest in the kidneys (0.66%) at 4 hours after administration. At 48 hours after administration only trace amounts (<0.01%) were detected in tissues. Up to 24 hours after dosing 0.4% of the administered activity was found in blood at both dose-levels (Williams and Blanchfield, 1975). Rats receiving orally 60 mg 14C-DBP/kg bw in DMSO did also not reveal significant retention in tissues (totally 14 tissues) 24 hours after dosing. No retention was seen in brain, heart, lung, spleen, testicles, prostate or thymus, 0.06% was found in liver, 0.02% in kidneys, 0.3% in muscle, 0.7% in adipose tissue, 1.53% in intestines, 0.01% in stomach and 0.02% in blood (Tanaka et al., 1978). | EC, 2003 (section 4.1.2.1.1) |
| **Type/Objective:** To determine distribution of DBP in rats after repeated oral doses.<br>**Conclusion:** No substantial accumulation in any tissue was seen.<br>**Method:** Twenty-four male Wistar rats (bw ca. 50 g) received ground rat chow mixed with 2% corn oil and 0.1% unlabeled DBP for up to 12 weeks. Twelve control rats were fed ground rat chow mixed with 2% corn oil. Eight treated rats and 4 control rats were killed after 4, 8 and 12 weeks. For the 4-week study the diets of 4 of the treated rats also contained 10 µCi of 14C-DBP/kg of feed; the other 4 treated rats in the 4-week study were fed this radioactive diet only for the last 24 hours. For the 8- and 12-week studies the diets contained 0.7 µCi 14C-DBP/kg of feed for the last 24 hours. At the end of the studies the rats were killed and organs and tissues (spleen, kidneys, adipose tissue, testes, skeletal muscle, heart, lungs, brain) removed and frozen until analyzed.<br>**Results:** No substantial accumulation in any tissue was seen (Williams and Blanchfield, 1975). | EC, 2003 (section 4.1.2.1.1) |
| **Type/Objective:** To measure distribution and retention of dermally applied DBP 7 days after dosing of rats.<br>**Conclusion:** Only 0.5-1.5% of the applied dose was found in tissues.<br>**Method:** Seven days after a dermal application under covered condition (plastic cap) of 43.7 mg/kg bw (157 µmol/kg bw) 14C-DBP in ethanol to the clipped skin (circular area with diameter of 1.3 cm) of male F344 rats (bw 180-220 g)<br>**Results:** Only 0.5-1.5% of the applied dose was found in tissues; adipose tissue (0.41%), skin (1.4%) and muscle (1.1%) contained most of the DBP remaining in the body; all other tissues combined (brain, lung, liver, spleen, small intestine, kidneys, testes, spinal cord, blood) contained less than 0.5%. Thirty three percent remained at the site of application (Elsisi et al., 1989). | EC, 2003 (section 4.1.2.1.1) |
| **Type/Objective:** To measure urinary metabolites of DBP in rats after oral dosing.<br>**Conclusion:** MBP was found, as well as MBP-glucuronide and other metabolites.<br>**Method:**<br>**Results:** After oral administration of DBP to rats mono-n-butyl phthalate (MBP) was detected in urine together with MBP glucuronide, various ω- and ω-1- | EC, 2003 (section 4.1.2.1.1) |

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| oxidation products of MBP (more polar ketones and carboxylates) and a small amount of free phthalic acid (Albro and Moore, 1974; Foster et al., 1982; Tanaka et al., 1978; Williams and Blanchfield, 1975) | |
| **Type/Objective:** To determine metabolites of DBP and excretion of metabolites in pregnant rats and to perform a pharmacokinetic study in pregnant rats<br>**Conclusion:** Fetal plasma levels of radioactivity from 14C-DBP were approximately half maternal plasma levels. Levels of several metabolites were reported, plasma MBP being themajor metabolite; no parent DBP was detected. The half-life for the maternal plasma MBP was similar in all doses (2.75-2.94 hours).<br>**Method:** In a pilot study, pregnant female rats were dosed at 100 mg/kg 14C-DBP by gavage on gestational day (gd) 20; virgin rats were similarly dosed on the same day. Samples were collected at 24 hours for the pregnant rats and 2 hours for the virgin rats. In a pharmacokinetic study of DBP in pregnant Sprague Dawley, rats given a single dose (50, 100, or 250 mg/kg) of DBP by gavage on gd 20, and analyzed maternal and fetal plasma and amniotic fluid samples by HPLC.<br>**Results:** In virgin rats, the majority (85%) of the dose was excreted within 24 hours, with 77% in the urine, 7% in the feces, and 0.008% remaining in the carcass. Using data from Saillenfait et al. (1998), 2 hours is thought to be the time of peak blood concentration. In the pregnant rats, Fennell et al. found similar concentrations of radioactivity in the plasma and carcass (329 and 357 μM, respectively). The fetal plasma concentration is approximately half that of the maternal plasma concentration (182 and 329 μM, respectively). Analysis of the virgin rat urine by HPLC showed a peak identified as MBP at 2 hours, which accounted for approximately 18% of the 14C label in the urine, and a peak identified as MBP-glucuronide, accounting for 61% of the 14C label in the urine. The maternal and fetal plasma were analyzed by shielded hydrophobic phase column mass spectrometry. Metabolites found in the urine and plasma were phthalic acid (1.6-2.6% urine; 0.7% plasma), MBP (12-31% urine; 77.1% plasma), MBP-glucuronide (MBP-G) (53-69% urine; 19% plasma), mono-n-hydroxybutylphthalate (8-9% urine; 1.7% plasma), monobutanoic phthtalic acid (0.8-0.9% urine; 0.6% plasma), mono-nhydroxybutylphthalate glucuronide (2.5-2.9% urine; 0.2% plasma), and monobutanoic phthalic acid glucuronide or mono-1-hydroxybutan-2-one phthalic acid glucuronide (0.5- 1.0% urine; none detected in plasma). Parent DBP was not detectable in the urine or plasma.<br><br>In the pharmacokinetic study in pregnant rats, MBP and MBP-G were found in the maternal and fetal plasma for all doses, with MBP being the major metabolite. The maximum concentration (Cmax) in plasma for MBP was 3-4 fold higher than the Cmax for MBP-G. The half-life for the maternal plasma MBP was similar in all doses (2.75-2.94 hours), as was the half-life of MBP-G (2.89-3.52 hours). For the fetal plasma, Cmax for MBP ranged from 40% to 65% of maternal Cmax. Cmax for MBP-G was 30% to 110% of the maternal Cmax. The time to maximum concentration for both MBP and MBPG was achieved later in the fetal plasma than the maternal plasma at 0.5-3hours versus 0.5- 1hr, respectively. In the amniotic fluid, MBP reached its maximum at 4 hours for all doses and had a half-life of about 6 hours for the 100 and 250 mg/kg doses. MBP-G reached its maximum at 8 hours for all doses and only decreased slightly by 24 hours. | Fennell et al, 2004, as summarized in CPSC, 2010 |

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| **Type/Objective:** Pharmacokinetic study in pregnant rats given MBP i.v. on GD 19<br>**Conclusion:** Maternal serum levels of MBP in pregnant rats decreased by 80% within 2 hours after an i.v. dose.<br>**Method:** 24 pregnant Sprague Dawley rats received 10, 30, or 50 mg MBP/kg body weight (bw) by intravenous injection on gd 19.<br>**Results:** Serum levels of MBP were decreased by 80% within 2 hours and MBP-G was noted in the blood within 5 minutes. MBP levels returned to background levels in maternal serum by 24 hours. However, both MBP and MBP-G were higher in the fetus at 24 hours. The half-life of maternal MBP-G was found to be about 2 hours, and Cmax increased with dose and was non-linear. | Kremer et al, 2005, as summarized in CPSC, 2010 |
| **Type/Objective: R**etrospective human biomonitoring study of metabolites of DBP and other phthalates in 24-hour urine samples<br>**Conclusion:** Intake of DBP was high in several subjects. The cumulative effet of all phthalates together needs to be accounted for.<br>**Method:** In a retrospective human biomonitoring study we analyzed 24h urine samples taken from the German Environmental Specimen Bank for Human Tissues (ESBHum), which were collected from 634 subjects (predominantly students, age range 20-29 years, 326 females, 308 males) in 9 years between 1988 and 2003 (each n >or= 60), for the concentrations of primary and/or secondary metabolites of di-n-butyl phthalate (DnBP), di-iso-butyl phthalate (DiBP), butylbenzyl phthalate (BBzP), di(2-ethylhexyl) phthalate (DEHP) and di-iso-nonyl phthalate (DiNP). Based on the urinary metabolite excretion we estimated daily intakes of the parent phthalates and investigated the chronological course of the phthalate exposure. In over 98% of the urine samples metabolites of all five phthalates were detectable indicating a ubiquitous exposure of the German population to all five phthalates throughout the last 20 years.<br>**Results:** The median daily intakes in the subsets between 1988 and 1993 were quite constant for DnBP (approx. 7 microg/kg bw/d) and DEHP (approx. 4 microg/kg bw/d). However, from 1996 the median levels of both phthalates decreased continuously until 2003 (DnBP 1.9 microg/kg bw/d; DEHP 2.4 microg/kg bw/d). By contrast, the daily intake values for DiBP were slightly increasing over the whole time frame investigated (median 1988: 1.1 microg/kg bw/d; median 2003: 1.4 microg/kg bw/d), approximating the levels for DnBP and DEHP. For BBzP we observed slightly decreasing values, even though the medians as of 1998 levelled off at around 0.2 microg/kg bw/d. Regarding daily DiNP exposure we found continuously increasing values, with the lowest median being 0.20 microg/kg bw/d for the subset of 1988 and the highest median for 2003 being twice as high. The trends observed in phthalate exposure may be associated with a change in production and usage pattern. Female subjects exhibited significantly higher daily intakes for the dibutyl phthalates (DnBP p=0.013; DiBP p=0.004). Compared to data from US National Health and Nutrition Examination Surveys (NHANES) exposure levels of the dibutyl phthalates were generally higher in our German study population, while levels of BBzP were somewhat lower. Overall, for a considerable 14% of the subjects we observed daily DnBP intakes above the tolerable daily intake (TDI) value deduced by the European Food Safety Authority (EFSA) (10 microg/kg bw/d). However, the frequency of exceedance decreased during the years and was beneath 2% in the 2003 subset.<br>**Conclusion:** Even though transgressions of the exposure limit values of the EFSA and the US Environmental Protection Agency (US EPA) occurred only in a relatively small share of the subjects, one has to take into account the cumulative | Wittassek et al 2007 |

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| exposure to all phthalates investigated and possible dose-additive endocrine effects of these phthalates. | |
| **Type/Objective:** Comparison of metabolic profiles of DBP in rats and humans<br>**Conclusion:** MBP is an optimal biomarker of exposure to DBP regardless of species.<br>**Method:** In humans and in rats, DBP is metabolized to mono-n-**butyl phthalate** (MBP). MBP may also further oxidize to other metabolites of DBP. We studied the metabolic profiles of DBP in rats and humans to evaluate the similarities between the two species and between different exposure scenarios.<br>**Results:** In rats administered DBP by oral gavage, we identified MBP and three urinary oxidative metabolites of DBP: mono-3-oxo-n-**butyl phthalate**, mono-3-hydroxy-n-**butyl phthalate** (MHBP), and mono-3-carboxypropyl phthalate (MCPP). MBP, MHBP, and MCPP were also present in serum, albeit at lower levels than in urine. Statistically significant correlations ($p < 0.01$) existed between the concentrations of MBP and the concentrations of MHBP (Pearson correlation coefficient $r = 0.82$ [urine] and $r = 0.96$ [serum]) and MCPP ($r = 0.77$ [urine] and $r = 0.97$ [serum]). However, the concentrations of these metabolites in urine collected 6 h after dosing and in serum 24 h after dosing were not correlated, suggesting continuous metabolism of DBP and/or individual differences among rats. Serum DBP metabolite concentrations increased with the dose, whereas urinary concentrations did not. We also identified MBP, MHBP, and MCPP in the urine of four men exposed to DBP bytaking a prescription medication containing DBP, and MBP and MCPP in 94 adults with no documented exposure to DBP. In the human samples, we observed statistically significant correlations ($p < 0.01$) among the urinary concentrations of MBP and MCPP, although the correlation was stronger for the four exposed men ($r = 0.99$) than for the adults without a documented exposure to DBP ($r = 0.70$). Our results suggest that regardless of species and exposure scenario, MBP, the major DBP metabolite, is an optimal biomarker of exposure to DBP. In addition to MBP, MCPP and MHBP may be adequate biomarkers of exposure to DBP in occupational settings orin potential high-exposure scenarios. | Silva et al, 2007 |
| **Type/Objective:** Our aim was to investigate the relationships between consumers' exposure to phthalates through food, consumers' interest in a natural and healthy diet, risk perception of food chemicals, and consumers' diet patterns.<br>**Conclusion:** This study shows that even those consumers who express strong interest in natural food and low acceptance of food chemicals, and who try to make respective food choices, are exposed to contaminants such as phthalates.<br>**Method:** We collected data through a mail survey in the adult Swiss-German population ($N = 1,200$). We modeled exposure to di(2-ethylhexyl) phthalate (DEHP), **dibutyl phthalate** (DBP), benzyl **butyl phthalate** (BBP), and diethyl phthalate (DEP) based on a food frequency questionnaire and phthalate concentrations reported from food surveys. Using rating scales, we assessed risk perceptions of chemicals in food and interest in a natural and healthy diet. Higher risk perceptions and higher natural and healthy diet interest were associated with higher daily doses of DEHP, BBP, and DEP.<br>**Results:** No health risk from phthalates in food was identified for the vast majority of the population. Four consumers' diet clusters were discerned, with differences in phthalate exposure, risk perceptions, and interest in a natural and healthy diet. | Dickson et al, 2009 |
| **Type/Objective:** Determination of levels of phthalates and metabolites in semen of 99 healthy volunteers | Han et al, 2009 |

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| **Conclusion:** These findings suggest the detection of phthalates in healthy human semen might require further investigation for effects on human fertility.<br>**Method:** Levels of the phthalates such as di(2-ethylhexyl) phthalate (DEHP), mono(2-ethylhexyl) phthalate (MEHP, a major metabolite of DEHP), di-n-**butyl phthalate** (DBP), mono-n-**butyl phthalate** (MBP, a major metabolite of DBP), and phthalic acid (P, (a common metabolite of phthalates, including DEHP and DBP) were determined in the semen samples of 99 healthy volunteers without known prior medicosurgical history. Samples were obtained from young men (age 20-25 yr) who visited a clinic, and the semen concentrations of phthalates were measured using ultra-performance liquid chromatography (UPLC) and tandem mass spectrometry (MS/MS).<br>**Results:** UPLC/MS/MS showed that mean concentrations in semen samples were 1.07 microg/ml for MEHP, 0.61 microg/ml for DEHP, 0.39 microg/ml for PA, 0.06 microg/ml for MBP, and 0.003 microg/ml for DBP. The concentration of MEHP (the metabolite of DEHP) was highest, and the concentrations of the metabolites including MEHP, MBP, and PA were higher than actual concentrations of parent DEHP and DBP. | |
| **Type/Objective:** The aim of this study was to determine kinetical data in humans after the application of a drug containing 3600 microg of DnBP and to quantify main metabolites of DnBP and DiBP with and without glucuronidase treatment.<br>**Conclusion:** Since an uptake of 3600 microg in only one capsule is already above the tolerable daily intake (TDI) for DnBP of 10 microg/kg b.w. from a preventive health protection DnBP should be replaced in medical drugs.<br>**Method:** One capsule containing 3600 microg of DnBP was given to each of to 17 volunteers.<br>**Results:** 78% (median of 2248 microg of total MnBP) of administered DnBP was found within 24h in urine. After 24h the levels of MnBP in urine were comparable to concentrations before administration showing a fast elimination. In contrast to controls in all urine samples collected within 24h after the administration of the drug free MnBP was observed with a median of 4% of total MnBP. In controls total MnBP and MiBP were found in median concentration of 23 microg/24h and about 50 microg/24h, respectively and therefore environmental exposure to DnBP is only 1% compared to medication. | Seckin et al, 2009 |
| **Type/Objective:** Repeated doses of di-n-butyl phthalate (DBP) from gestation day (GD) 12 to 19 disrupt testosterone synthesis and male sexual development in the fetal rat. Currently little is known about the disposition of DBP metabolites, such as monobutyl phthalate (MBP) and its glucuronide conjugate (MBP-G), during gestation after repeated exposure to DBP in rats.<br>**Conclusion:** MBP kinetics in fetal testes allows direct comparison of active metabolite concentrations and testosterone response in the fetal testes.<br>**Method:** In order to gain a better understanding of the effect of repeated dosing on maternal and fetal metabolism and distribution, pregnant Sprague-Dawley rats were given a single dose of 500 mg/kg DBP on GD 19 or daily doses of 50, 100, and 500 mg/(kg day) from GD 12 to 19 via corn oil gavage.<br>**Results:** Dose-response evaluation revealed a non-linear increase in maternal and fetal plasma concentrations of MBP. Maternal and fetal MBP levels were slightly lower in animals after 8 days of dosing at 500 mg/(kg day). Fetal plasma MBP levels closely followed maternal plasma, while the appearance and elimination of MBP-G in fetal plasma were significantly delayed. MBP-G accumulated over time in the amniotic fluid. Inhibition of testosterone was rapid in fetal testes when exposed to DBP (500 mg/(kg day)) on GD 19. Within 24h, the level of inhibition | Clewell et al, 2009 |

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| in the fetus was similar between animals exposed to a single or multiple daily doses of 500 mg/(kg day). Examination of testosterone time-course data indicates a rapid recovery to normal levels within 24h post-dosing at DBP doses of 50 and 100 mg/(kg day), with a rebound to higher than normal concentrations at later time-points. | |
| **Type/Objective:** Estimating the chronological sequences of the phthalate exposure, we performed a retrospective human biomonitoring study by investigating the metabolites of the five most prominent phthalates in urine.<br>**Conclusion: W**e found decreases of the internal human exposure for legally restricted phthalates whereas the exposure to their substitutes increased. Future investigations should verify these trends. This is of increasing importance since the European Commission decided to require ban or authorization from 1.1.2015 for DEHP, DnBP, DiBP and BzBP according to REACh Annex XIV.<br>**Method:** 24h-urine samples from the German Environmental Specimen Bank (ESB) collected from 240 subjects (predominantly students, age range 19-29 years, 120 females, 120 males) in the years 2002, 2004, 2006 and 2008 (60 individuals each), were analysed for the concentrations of mono-n-**butyl phthalate** (MnBP) as metabolite of di-n-**butyl phthalate** (DnBP), mono-iso-**butyl phthalate** (MiBP) as metabolite of di-iso-**butyl phthalate** (DiBP), mono-benzyl phthalate (MBzP) as metabolite of butylbenzyl phthalate (BBzP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP), mono-(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP) and mono-(2-carboxymethyl hexyl) phthalate (2cx-MMHxP) as metabolites of di(2-ethylhexyl) phthalate (DEHP), monohydroxylated (OH-MiNP), monooxidated (oxo-MiNP) and monocarboxylated (cx-MiNP) mono-iso-nonylphthalates as metabolites of di-iso-nonyl phthalates (DiNP). Based on the urinary metabolite excretion, together with results of a previous study, which covered the years 1988-2003, we investigated the chronological sequences of the phthalate exposure over two decades.<br>**Results:** In more than 98% of the urine samples metabolites of all five phthalates were detectable indicating a ubiquitous exposure of people living in Germany to all five phthalates throughout the period investigated. The medians in samples from the different years investigated are 65.4 (2002), 38.5 (2004), 29.3 (2006) and 19.6 µg/l (2008) for MnBP, 31.4 (2002), 25.4 (2004), 31.8 (2006) and 25.5 µg/l (2008) for MiBP, 7.8 (2002), 6.3 (2004), 3.6 (2006) and 3.8 µg/l (2008) for MBzP, 7.0 (2002), 5.6 (2004), 4.1 (2006) and 3.3 µg/l (2008) for MEHP, 19.6 (2002), 16.2 (2004), 13.2 (2006) and 9.6 µg/l (2008) for 5OH-MEHP, 13.9 (2002), 11.8 (2004), 8.3 (2006) and 6.4 µg/l (2008) for 5oxo-MEHP, 18.7 (2002), 16.5 (2004), 13.8 (2006) and 10.2 µg/l (2008) for 5cx-MEPP, 7.2 (2002), 6.5 (2004), 5.1 (2006) and 4.6 µg/l (2008) for 2cx-MMHxP, 3.3 (2002), 2.8 (2004), 3.5 (2006) and 3.6 µg/l (2008) for OH-MiNP, 2.1 (2002), 2.1 (2004), 2.2 (2006) and 2.3 µg/l (2008) for oxo-MiNP and 4.1 (2002), 3.2 (2004), 4.1 (2006) and 3.6 µg/l (2008) for cx-MiNP. The investigation of the time series 1988-2008 indicates a decrease of the internal exposure to DnBP by the factor of 7-8 and to DEHP and BzBP by the factor of 2-3. In contrast, an increase of the internal exposure by the factor of 4 was observed for DiNP over the study period. The exposure to DiBP was found to be stable. | Göen et al, 2011 |
| **Type/Objective:** Metabolic profile of DBP and other phthalates by human liver microsomes<br>**Conclusion:** Results suggest that the hydrolysis activities of diester phthalates by human liver microsomes depend on the chemical structure, and that the | Hanioka et al, 2012 |

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| metabolism profile may relate to diester phthalate toxicities, such as hormone disruption and reproductive effects.<br>**Method:** We examined the hydrolysis of DBP, butylbenzyl phthalate (BBzP) and di(2-ethylhexyl) phthalate (DEHP) in human liver microsomes.<br>**Results:** These diester phthalates were hydrolyzed to monoester phthalates (mono-n-**butyl phthalate** (MBP) from DBP, mono-n-**butyl phthalate** (MBP) and monobenzyl phthalate (MBzP) from BBzP, and mono(2-ethylhexyl) phthalate (MEHP)) by human liver microsomes. DBP, BBzP and DEHP hydrolysis showed sigmoidal kinetics in V-[S] plots, and the Hill coefficient (n) ranged 1.37-1.96. The S(50), V(max) and CL(max) values for DBP hydrolysis to MBP were 99.7 μM, 17.2nmolmin(-1)mg(-1) protein and 85.6 μL min(-1)mg(-1) protein, respectively. In BBzP hydrolysis, the values of S(50) (71.7 μM), V(max) (13.0nmolmin(-1)mg(-1) protein) and CL(max) (91.3 μL min(-1)mg(-1) protein) for MBzP formation were comparable to those of DBP hydrolysis. Although the S(50) value for MBP formation was comparable to that of MBzP formation, the V(max) and CL(max) values were markedly lower (<3%) than those for MBzP formation. The S(50), V(max) and CL(max) values for DEHP hydrolysis were 8.40 μM, 0.43 nmol min(-1)mg(-1) protein and 27.5 μL min(-1)mg(-1) protein, respectively. The S(50) value was about 10% of DBP and BBzP hydrolysis, and the V(max) value was also markedly lower (<3%) than those for DBP hydrolysis and MBzP formation for BBzP hydrolysis. The ranking order of CL(max) values for monoester phthalate formation in DBP, BBzP and DEHP hydrolysis was BBzP to MBzP≥DBP to MBP>DEHP to MEHP>BBzP to MBP. | |
| **Type/Objective:** Metabolic profile in one man of ingested DBP and diisobutylphthalte<br>**Conclusion:** This study provides basic human metabolism and toxicokinetic data for two phthalates that have to be considered human reproductive toxicants and that have been shown to be omnipresent in humans. Peak concentrations of MBP and other metabolites was at 2-4 hours after an oral dose, followed by a monotonic decline.<br>**Method:** An individual (male, 36 years, 87 kg) ingested two separate doses of di-n-**butyl phthalate** (DnBP) and diisobutyl phthalate (DiBP) at a rate of ~60 μg/kg. Key monoester and oxidized metabolites were identified and quantified in urine continuously collected until 48 h post-dose.<br>**Results:** For both DnBP and DiBP, the majority of the dose was excreted in the first 24 h (92.2 % of DnBP, 90.3 % of DiBP), while only <1 % of the dose was excreted in urine on day 2. In each case, the simple monoesters were the major metabolites (MnBP, 84 %; MiBP, 71 %). For DnBP, ~8 % was excreted as various side chain oxidized metabolites. For DiBP, approximately 20 % was excreted mainly as the oxidized side chain metabolite 2OH-MiBP, indicating that the extent of oxidative modification is around 2.5 times higher for DiBP than for DnBP. All DnBP and DiBP metabolites reached peak concentrations between 2 and 4 h post-exposure, followed by a monotonic decline. For DnBP metabolites, the elimination halftime of MnBP was 2.6 h; longer elimination halftimes were estimated for the oxidized metabolites (2.9-6.9 h). For DiBP metabolites, MiBP had the shortest halftime (3.9 h), and the oxidized metabolites had somewhat longer halftimes (4.1 and 4.2 h). Together with the simple monoesters, secondary oxidized metabolites are additional and valuable biomarkers of phthalate exposure. | Koch et al, 2012 |

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| **Type/Objective:** To examine the reliability of urinary phthalate levels in exposure classification by comparing the inter- and intrasubject variation of urinary phthalate metabolite levels.<br>**Conclusion:** The only slightly higher ICCs for 24-h pools compared to first-morning and spot urine samples does not seem to justify the extra effort needed to collect 24-h pools.<br>**Method:** Thirty-three young healthy men each collected two spot, three first-morning, and three 24-h urine samples during a 3-month period. Samples were analyzed for the content of 12 urinary metabolites of 7 different phthalates. Variability was assessed as intraclass correlation coefficients (ICC).<br>**Results:** For the metabolites of diethyl-, dibutyl-, and butylbenzyl-phthalates moderate ICCs were observed in all three sample types, albeit highest in 24-h urine (0.51-0.59). For the metabolites of di(2-ethylhexyl) phthalate and di-iso-nonyl phthlates lower ICCs (0.06-0.29) were found. These low ICCs indicate a high risk of misclassification of exposures for these two phthalates in population studies and hence an attenuation of the power to detect possible exposure-outcome associations. | Frederiksen et al, 2012 |
| **Type/Objective:** Several plasticizers have been illegally used as clouding agents to increase dispersion of aqueous matrix in beverages. This study thus develops a rapid and validated analytical method by ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) for the evaluation of pharmacokinetics of DBP in free moving rats.<br>**Conclusion:** The pharmacokinetic behavior demonstrated that DBP was quickly degraded within 2 h, suggesting a rapid metabolism low fecal cumulative excretion in the rat.<br>**Method:** The UPLC-MS/MS system equipped with positive electrospray ionization (ESI) source in multiple reaction monitoring (MRM) mode was used to monitor m/z 279.25→148.93 transitions for DBP. The limit of quantification for DBP in rat plasma and feces was 0.05 μg/mL and 0.125 μg/g, respectively.<br>**Results:** The pharmacokinetic results demonstrate that DBP appeared to have a two-compartment model in the rats; the area under concentration versus time (AUC) was 57.8 ± 5.93 min μg/mL and the distribution and elimination half-life (t(1/2,α) and t(1/2,β)) were 5.77 ± 1.14 and 217 ± 131 min, respectively, after DBP administration (30 mg/kg, i.v.). About 0.18% of the administered dose was recovered from the feces within 48 h. | Chang et al, 2013 |
| **Type/Objective:** Presentation of phthalate metabolites biomonitoring data from a nationally-representative Canadian survey.<br>**Conclusion:** Factors associated with higher levels of phthalates and metabolites are discussed.<br>**Method:** In the Canadian Health Measures Survey 2007-2009, 11 phthalate metabolites, namely, MMP, MEP, MnBP, MBzP, MCHP, MCPP, MEHP, MEOHP, MEHHP, MnOP, and MiNP were measured in urine samples of 6-49 year old survey respondents (n=3236).<br>**Results:** The metabolites MEP, MnBP, MBzP, MCPP, MEHP, MEOHP and MEHHP were detected in >90% of Canadians while MMP, MCHP, MnOP and MiNP were detected in <20% of the Canadian population. Step-wise regression analyses were carried out to identify important predictors of volumetric concentrations (μg/L) of the metabolites in the general population. Individual multiple regression models with covariates age, sex, creatinine, fasting status, and the interaction terms age×creatinine, age×sex and fasting status×creatinine were constructed for MEP, MnBP, MBzP, MCPP, MEHP, MEOHP and MEHHP. The | Saravanabhavan et al, 2013 |

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| least square geometric mean (LSGM) estimates for volumetric concentration (μg/L) of the metabolites derived from respective regression models were used to assess the patterns in the metabolite concentrations among population sub-groups. The results indicate that children had significantly higher urinary concentrations of MnBP, MBzP, MEHP, MEHHP, MEOHP and MCPP than adolescents and adults. Moreover, MEP, MBzP, MnBP and MEOHP concentrations in females were significantly higher than in males. We observed that fasting status significantly affects the concentrations of MEHP, MEHHP, MEOHP, and MCPP metabolites analyzed in this study. Moreover, our results indicate that the sampling time could affect the DEHP metabolite concentrations in the general Canadian population. | |
| **Type/Objective:** One goal of this model application was to confirm the validity of the calibrated pharmacokinetic models - their validity would be demonstrated if a profile of intakes could be found which adequately duplicated the metabolite concentrations measured in the urine. A second goal was to study patterns of exposure for a group of 5 people.<br>**Conclusion:** Observed intake of phthalates did not appear to be associated with self-reported activities.<br>**Method:** In a published controlled dosing experiment, a single individual consumed 5mg each of labeled di-n-**butyl phthalate** (DnBP) and diisobutyl phthalate (DiBP) on separate occasions and tracked metabolites in his blood and urine over 48h. Data from this study were used to structure and calibrate simple pharmacokinetic (PK) models for these two phthalates, which predict urine and blood metabolite concentrations with a given phthalate intake scenario (times and quantities). The calibrated models were applied to a second published experiment in which 5 individuals fasted over the course of a 48-h weekend (bottled water only), and their full urine voids were captured and measured for DnBP and DiBP metabolites.<br>**Results:** It was found that all metabolites could be duplicated very well with individual-specific "best-fit" intake scenarios, with one exception. It appears that the model predicted much lower concentrations of the metabolite, 3carboxy-mono-propylphthalate (MCPP), than were observed in all individuals. Modeled as a metabolite of DnBP, this suggests that DnBP was not the major source of MCPP in the urine. For all 5 individuals, the reconstructed dose profiles of the two phthalates were similar: about 6 small bolus doses per day and an intake of about 0.5μg/kg-day. The intakes did not appear to be associated with diary-reported activities (personal hygiene and medication) of the participants. The modeled frequent intakes suggested one (or both) of two possibilities: ongoing exposures such as an inhalation exposure, or no exposure but rather an ongoing release of body stores of the phthalate metabolites from past exposures. | Lorber and Koch, 2013 |
| **Type/Objective:** Dermal exposure via skin care products, soil, and dust is a main route for phthalate delivery. We had explored the effect of topically-applied phthalates on skin absorption and toxicity.<br>**Conclusion:** DBP was absorbed across human skin. Additional observations are discussed.<br>**Method:** Immunohistology, functional proteomics, and Western blotting were employed as methodologies for validating phthalate toxicity.<br>**Results:** Among 5 phthalates tested, di(2-ethylhexyl)phthalate (DEHP) showed the highest skin reservoir. Only diethyl phthalate (DEP) and dibutyl phthalate (DBP) could penetrate across skin. Strat-M(®) membrane could be used as permeation barrier for predicting phthalate penetration through skin. The | Pan et al, 2014 |

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| accumulation of DEHP in hair follicles was ~15nmol/cm(2), which was significantly greater than DBP and DEP. DBP induced apoptosis of keratinocytes and fibroblasts via caspase-3 activation. This result was confirmed by downregulation of 14-3-3 and immunohistology of TUNEL. On the other hand, the HSP60 overexpression and immunostaining of COX-2 suggested inflammatory response induced by DEP and DEHP. The proteomic profiling verified the role of calcium homeostasis on skin inflammation. Some proteins investigated in this study can be sensitive biomarkers for dermal toxicity of phthalates. These included HSPs, 14-3-3, and cytokeratin. | |

## BACKGROUND INFORMATION FOR TABLE 34: CARCINOGENICITY

| Summary of Study | Reference |
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| **Type/Objective:** sister chromatid exchange<br>**Conclusion:** Marginal response in a pseudodiploid Chinese hamster cell line (Don) in the absence of a metabolic activation system | Abe and Sasaki, 1977 |
| **Type/Objective:** reverse mutations in *Saccharomyces cerevisiae*<br>**Conclusion:** DBP did not induce mutations in the presence or absence of a metabolic activation system. | Shahin and VonBorstel, 1977 |
| **Type/Objective:** Mutagenicity in Salmonella strains<br>**Conclusion:** DBP was not mutagenic in Salmonella strains TA98, TA100, TA1535, TA1537 in the presence or absence of a liver S-9 metabolic activation system. | Zeiger et al, 1985 |
| **Type/Objective:** In vivo assay in mammals for peroxisome activation<br>**Conclusion:** NOAEL was not established since lowest dose caused increased activities of peroxisome associated enzymes.<br>**Method:** In a 3-week dietary study in M and F F344 rats doses of 0.6, 1.2 and 2.5% in the diet (ca. 600, 1,200, and 2,100 mg/kg/bw) were given.<br>**Results:** A NOAEL could not be established because the lowest dose of 0.6% (ca. 600 mg/kg bw) caused increased activities of peroxisome associated enzymes (PCoA, LAH-11 and LAH-12). In addition increased liver weights and decreased serum triglyceride and cholesterol levels were found at this dose-level. | Barber et al, 1987, as summarized in EC, 2003 |
| **Type/Objective:** To determine dietary dose needed for hepatic peroxisome proliferation in rat.<br>**Conclusion:** NOAEL for 3-month study was ~152 mg/kg.<br>**Method:** In a 3-month dietary toxicity study in Wistar rats groups of 3 m and 3 f animals received 400, 2,000, or 10,000 mg DBP/kg of diet (~ ca. 30, 152 and 752 mg/kg bw).<br>**Results:** At the end of the treatment period peroxisomal proliferation in the liver was determined by a histochemical method, measuring number and/or size of peroxisomes. NOAEL for peroxisomal proliferation appeared to be 2,000 mg/kg of diet (ca. 152 mg/kg bw). | Kaufmann, 1992, as summarized in EC, 2003 |
| **Type/Objective:** To determine dietary dose needed for hepatic peroxisome proliferation in rat.<br>**Conclusion:** NOAEL for the induction of peroxisomal associated enzymes is 200 mg/kg of diet (19.9 mg/kg bw)<br>**Method:** 2-week dietary study in male Wistar rats given 20, 60, 200, 600 and 2,000 mg DBP/kg of diet (equal to 1.1, 5.2, 19.9, 60.6 and 212.5 mg/kg bw).<br>**Results:** The lowest NOAEL for peroxisome proliferation was found in this study. NOAEL for PCoA activity was 600 mg/kg of diet (60.6 mg/kg bw) and for LAH-11 and LAH-12 activity 200 mg/kg of diet (19.9 mg/kg bw). The overall NOAEL for the induction of peroxisomal associated enzymes is 200 mg/kg of diet (19.9 mg/kg bw). | Jansen et al, 1993, as summarized in EC, 2003 |

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| **Type/Objective:** Mutagenicity in Salmonella<br>**Conclusion:** DBP was not mutagenic in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation | NTP, 1995 |
| **Type/Objective:** Mouse lymphoma assay<br>**Conclusion:** DBP did induce mutations in L5178Y mouse lymphoma cells treated without metabolic activation. | NTP, 1995 |
| **Type/Objective:** Micronucleus assay<br>**Conclusion:** In peripheral blood samples obtained from male and female mice at the end of NTP's 13-week study, frequencies of micronucleated normochromatic erythrocytes were similar between exposed and control mice. | NTP, 1995 |
| **Type/Objective:** Mouse lymphoma assay<br>**Conclusion:** DBP produced significant increases in the frequency of mutations in the mouse lymphoma assay using L5178Y cells in the presence but not in the absence of an S-9 Arochlor-induced rat liver activation system. | Barber et al, 2000 |
| **Type/Objective:** Transformations in cell line<br>**Conclusion:**DBP did not increase the frequency of transformations in Balb/3T3 cells. | Barber et al, 2000 |
| **Type/Objective:** Microgel electrophoresis assay to detect single-strand breaks in the DNA (Comet assay) of human epithelia and human mucosal cells derived from biopsies harvested during surgery of the oropharynx and the inferior nasal turbinate, respectively.<br>**Conclusion:** The results demonstrate genotoxic effects of phthalates on human mucosal cells of the upper aerodigestive tract, in contrast to earlier findings in animal models.<br>**Method:** The alkaline version of the microgel electrophoresis assay was used to detect single-strand breaks in the DNA following incubation with dibutylphthalate (DBP) and diisobutylphthalate (DiBP).<br>**Results:** DNA damage was induced by both DBP and DiBP in oropharyngeal and nasal mucosa, though the effect of DiBP was more pronounced than that of DBP. Nasal mucosa proved to be more sensitive than oropharyngeal epithelia. | Kleinsasser et al, 2000 |
| **Type/Objective:** DBP and DiBP were genotoxic in human mucosal cells of the upper aerodigestive tract in a Comet assay. Furthermore, higher genotoxic sensitivities of patients with squamous cell carcinomas of either the larynx or the oropharynx compared to non-tumor patients were described. It was the aim of the present study to determine whether there is a correlation between the genotoxic sensitivities to DBP and its isomer DiBP in either mucosal cells or lymphocytes.<br>**Conclusion:** DBP and DiBP were positive for genotoxicity in a Comet assay using in human mucosal cells from the oropharynx and in lymphocytes.<br>**Method:** The single-cell microgel electrophoresis assay (Comet assay) was applied to detect DNA strand breaks in human epithelial cells of the upper aerodigestive tract (n=132 specimens). Human mucosa was harvested from the oropharynx in non-tumor patients and patients with squamous cell carcinomas of the oropharynx. Laryngeal mucosa of patients with laryngeal squamous cell carcinomas was harvested as well. Peripheral lymphocytes (n=49 specimens) were separated from peripheral blood. Xenobiotics investigated were DBP, DiBP, and N'methyl-N'-nitro-N-nitrosoguanidine (MNNG) as positive control, respectively.<br>**Results:** Genotoxicity was found for DBP and DiBP in epithelial cells and lymphocytes ($P<0.001$). MNNG caused severe DNA damage. In analyzing DBP and DiBP results, genotoxic impacts in mucosal cells showed an intermediate correlation ($r=0.570$). Correlation in lymphocytes was the same ($r=0.570$). | Kleinsasser et al, 2001 |

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| Phthalates have been investigated as a potential health hazard for a variety of reasons, including possible xenoestrogenic impact, peroxisome proliferation, and membrane destabilization. | |
| **Type/Objective:** The granulin (grn) precursor gene and p130 gene were previously identified as sex steroid regulated genes in the rat hypothalamus that may be involved in sexual differentiation of the rat brain. In an effort to correlate serum sex steroid level and hypothalamic gene expression, rats were given DBP and tested for sex hormones and gene expression.<br>**Conclusion:** The authors conclude that the increase in grn expression in female rats may be due to the DBP estrogenic properties, and the p130 gene increase in male rats may be due to DBP's mild androgenic properties due to the non-dose dependent nature of the increases.<br>**Method:** Authors used pregnant Wistar rats that were fed 20, 200, 2000, or 10,000 ppm DBP from gd 15 to weaning. On pnd 7, serum testosterone and estrodiol levels and gene expression of grn and p130 were evaluated.<br>**Results:** DBP (2,000 ppm) decreased estradiol in female rats, but the serum concentration of testosterone was unaffected and estradiol was unaffected at the other concentrations. At pnd 7, female pups showed an increase in grn gene expression with 2000 and 10,000 ppm doses, but grn expression was unchanged in male rats at these doses. The p130 gene expression was increased at the lower doses (20 and 200 ppm) in male rats, and was unaffected in female rats. | Lee et al, 2006, as summarized in CPSC, 2010 |
| **Type/Objective:** Biological effects of phthalates are believed to be mediated in part by peroxisome proliferator-activated receptors (PPARs). Evaluations of the monoester metabolites of phthalates as ligands toward PPARs have been investigated. This study evaluated other metabolites, including oxidized derivatives.<br>**Conclusion:** Results might imply indirect PPAR-mediated mechanisms that lead to observed biological effects such as peroxisome proliferation.<br>**Method:** We have evaluated the PPAR ligand activities of these PE derivatives by in vitro coactivator recruiting assay.<br>**Results:** Mono(2-ethyl-5-hydroxyhexyl)phthalate, the most abundant metabolite of di-(2-ethylhexyl)phthalate (DEHP), was less active than mono(ethylhexyl)phthalate (MEHP) as a PPAR ligand. Other derivatives oxidized at the alkyl group and benzene ring of DEHP, MEHP, dibutyl phthalate and its monoester were also investigated and some affected PPAR activities. Unexpectedly, MEHP as well as its further oxidized metabolite did not show clear activity for PPARalpha, although MEHP is believed to interact with PPARalpha. | Kusu et al, 2008 |
| **Type/Objective:** Peroxisome proliferators (PPs)-induced DNA hypomethylation has been proposed as a mechanism of the toxicity of phthalates, including carcinogenic action. The effect of DBP, a known peroxisome proliferator, on the methylation level of the c-myc promoter region in rat liver was studied.<br>**Conclusion:** Authors hypothesize that DBP-induced demethylation of the c-myc gene was an active mechanism, not associated with DNMTs activity and DNA replication.<br>**Method:** Changes in the methylation status of the c-myc gene were correlated with changes in DNA synthesis, DNA methyltransferase (DNMTs) activity and liver weight. Male Wistar rats received DBP in one, three or fourteen daily oral doses of 1800 mg/kg body weight (b.w.) x day(-1) (this dose is close to the dose that increases the numbers of peroxisomes in male Wistar rats).<br>**Results:** DBP decreased the methylation of the c-myc gene. Cytosine hypomethylation in the analyzed CpG sites of the c-myc gene promoter occurred | Kostka et al, 2010 |

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| during the whole period of study, although after 14 doses of DBP the difference from control was only on the borderline of significance (p = 0.066). An increase in DNA synthesis was only observed after 24 hours of treatment with DBP, and it preceded liver growth. | |
| **Type/Objective:** DBP is reported to inhibit estrogen receptor (ER)-mediated gene expression and to interfere with normal fetal development of the male reproductive system. Hexabromocyclododecane (HBCD or HBCDD) is one of the brominated flame retardants (BFRs) known to cause endocrine disruption with toxicity of the nervous system. The estrogenic effects of DBP and HBCD were examined in an ovarian cancer cell line.<br>**Conclusion:** Our results suggest that DBP and HBCD have sufficient potency to disrupt the endocrine system and to stimulate cell growth in ER-positive cancer cells.<br>**Method:** In the present study, the estrogenic effects of DBP and HBCD were examined in an ovarian cancer cell line, BG-1, expressing high levels of ER via MTT assay and semi-quantitative reverse-transcription PCR.<br>**Results:** Treatment with DBP (10(-8)-10(-5) M) or HBCD (2 x 10(-8) -2 x 10(-6) M) resulted in increased cell proliferation of BG-1 cells as observed with 17-β estradiol (E2). In addition, both DBP and HBCD upregulated the expression levels of cell cycle-regulatory genes, such as cyclin D and cyclin-dependent kinase-4 (cdk-4), which are downstream target genes of ER, at 6 h after treatment. However, the expression of the p21 gene was not altered by DBP or HBCD at any time as with E2. Taken together, these results suggest that DBP and HBCD are EDCs which have apparent estrogenic activities by stimulating the cell proliferation of BG-1 cells and by inducing the expression of cyclin D and cdk-4. | Park et al, 2011 |
| **Type/Objective:** To investigate the role of phthalates in the etiology of hormone-independent cancer.<br>**Conclusion:** Findings revealed a novel oncogenic mechanism of phthalates in breast cancer independent from their estrogenic activities and based on phthalate-induced AhR promoted tumorigenesis of estrogen receptor-negative breast cancer.<br>**Results:** Here we show that treatments with the phthalates n-butyl benzyl phthalate (BBP) and dibutyl phthalate (DBP) at 1 μM induced proliferation (BBP, 3.2-fold; DBP, 3.2-fold), migration (BBP, 2.6-fold; DBP, 2.6-fold), invasion (BBP, 2.7-fold; DBP, 3.1-fold), and tumor formation (EC(50): BBP, 0.12 μM; DBP, 0.22 μM) in estrogen receptor (ER)-negative breast cancer cells (MDA-MB-231). We further demonstrate that phthalates stimulated the cell surface aryl hydrocarbon receptor (AhR) and triggered the downstream cyclic AMP (cAMP)-PKA-CREB1 signaling cascade. The pathway led to increased expression of HDAC6, which facilitated nuclear assembly of the β-catenin-LEF1/TCF4 transcriptional complex and transactivation of the c-Myc oncogene. This nongenomic pathway emanated from the phthalate-induced AhR promoted tumorigenesis of ER-negative breast cancer. | Hsieh et al, 2011 |
| **Type/Objective:** We investigated the relationship between DBP-induced hypomethylation of the c-Myc promoter region and the expression of c-Myc and DNMT1 genes (at messenger RNA (mRNA) and protein level) in the rat liver.<br>**Conclusions:** Based on our previous and present results: (1) DBP exerted biological activity through epigenetic modulation of c-Myc gene expression; (2) it seems possible that DBP-induced active demethylation of c-Myc gene through mechanism(s) linked to generation of reactive oxygen species by activated c-Myc; and (3) control of DNA replication was not directly dependent on c-Myc transcriptional activity and we attribute this finding to DNMT1gene expression | Urbanek-Olejnik et al, 2013 |

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| which was tightly coordinated with DNA synthesis.<br>**Method:** Male Wistar rats received DBP in 1, 3, or 14 daily doses of 1800 mg $kg^{-1}$ body weight. Levels of DNMT1, c-Myc mRNA, and proteins were detected using real-time polymerase chain reaction and Western blot analysis, respectively.<br>**Results:** Our findings indicate that DBP caused an increase in mRNA levels of c-Myc at all time points. The results showed that protein levels of c-Myc in rat liver also increased significantly by DBP treatment, which were more pronounced at last time point (after 14 doses). Furthermore, overexpression of DNMT1gene have been found after one dose of DBP, which was confirmed at the protein level by Western blot analysis. Reduced levels of DNMT1mRNA and proteins (3 and 14 doses) were coordinated with depletion DNA synthesis (reported previously). | |
| **Type/Objective:** To explore the effect and pathway of phthalates on the growth of MCF-7 breast cancer cells<br>**Conclusion:** The present study demonstrates that, even at a very low concentration, BBP, DBP, and DEHP were not only still capable of inducing a proliferative effect through the PI3K/AKT signaling pathway but also displaying estrogenic activity.<br>**Method:** MCF-7 cells were treated with benzyl **butyl phthalate** (BBP), di-n-**butyl phthalate** (DBP), and di-2-ethylhexyl phthalate (DEHP) (10(-10)-10(-4) mol/l). After incubation for 24, 48, 72, and 92 h, the cells were harvested and extracted for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The proteins involving proliferative and apoptotic pathways were evaluated by Western blot analysis.<br>**Results:** MTT assay revealed cell toxicity at more than 10(-5) mol/l for DEHP and at 10(-4) mol/l for both BBP and DBP in MCF-7 cells. Cell proliferation was significantly increased at 10(-8)-10(-5) mol/l of BBP and DBP, and at 10(-8)-10(-6) mol/l of DEHP treatment. Proliferating cell nuclear antigen (PCNA) was substantially increased in cultures with DEHP (10(-8)-10(-6) mol/l), BBP (10(-8)-10(-5) mol/l), and DBP (10(-7)-10(-5) mol/l). Obvious increases in PI3K, p-AKT, and PCNA were noted in cultures with 17β-estradiol, BBP, DBP, and DEHP. Estrogen receptor α expression was also notably increased in treatment with estradiol, BBP, DBP, and DEHP. | Chen and Chien, 2014 |
| **Type/Objective:** To evaluate the impact of DEHP and DBP on the proliferation of androgen-sensitive human prostate carcinoma LNCaP cells<br>**Conclusion:** Taken together, the presented data indicate that phthalates may exert long-term negative effects on the proliferation of prostate epithelial cells derived from the carcinoma model, which are, nevertheless, largely independent of the modulation of AR expression/activity, and which do not alter further processes associated with NED.<br>**Method:** we examined the impact of diethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP) on the proliferation of androgen-sensitive human prostate carcinoma LNCaP cells and related events.<br>**Results:** The results showed that both compounds were able to inhibit cell cycle progression in a dose-dependent manner. However, only DEHP was found to weakly reduce androgen receptor (AR) protein levels after long-term exposure, while only DBP partially inhibited expression of the prostate-specific antigen (KLK3) gene, a model AR transcriptional target. This indicated that inhibition of cell proliferation was likely independent of any AR modulations. Both phthalates induced suppression of cell proliferation, but none of them affected the levels of markers associated with neuroendocrine transdifferentiation (NED) in LNCaP cells. | Hrubá et al, 2014 |

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| **Type/Objective:** The distinct roles of estrogen receptors (ERs) related with androgen receptors (ARs) have been proposed in prostate cancer, while the involvement of transforming growth factor-β (TGF-β) has been reported in the progression of prostate cancer. In this study, we examined whether the TGF-β signaling pathway is associated with ER signaling in LNCaP prostate cancer cells, which express ERα, ERβ and ARs.<br>**Conclusion:** These results indicate that DBP may induce the growth of LNCaP prostate cancer by acting on the crosstalk between TGF-β and ER signaling pathways.<br>**Method:** We determined whether the exposure to phthalates may induce prostate cancer progression by affecting molecular crosstalk between ER and TGF-β signaling pathways. Cell viability was measured in LNCaP cells by MTT assay following treatment with di-n-buthyl phthalate (DBP). RT-PCR and immunoblot assay were performed to examine the expression levels of cell cycle-related genes and the TGF-β signaling cascade. A mouse xenograft model of prostate cancer was generated, and immunohistochemical and BrdU assay were carried out to determine the effect of DBP in this mouse model.<br>**Results:** DBP, a type of phthalate, was shown to promote LNCaP cell proliferation by upregulating the gene expression of c-myc and cyclin D1 and by downregulating the expression of p21. DBP significantly reduced the protein expression of p-smad similarly to E2. These regulations caused by DBP were reversed by ICI 182,780, an ER antagonist, indicating that DBP may affect crosstalk between TGF-β and ER signals. In an in vivo mouse model, tumor volume of mice exposed to DBP was increased. Number of cells in S phase of cell cycle was increased by DBP, while expression of p21 protein was reduced in the tissues of DBP-treated mice. | Lee et al, 2014 |

**BACKGROUND INFORMATION FOR TABLE 35A: ANIMAL STUDIES ON ADVERSE EFFECTS ON SEXUAL FUNCTION AND FERTILITY**

| Summary of Study | Reference |
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| **Type/Objective:** Fertility studies in Charles River COBS CD rats, performed under GLP conditions<br>**Conclusion:** The NOAEL for male fertility and embryotoxicity in this study is 500 mg/kg bw, the highest dose tested. The NOAEL in the female fertility study is 50 mg/kg bw study based on maternal toxicity (reduced weight gain) and embryotoxicity (reduced pup weight and, in male pups, testicular lesions and reduced testicular weight) at 500 mg/kg bw.<br>**Method:** Male or female rats were exposed to DBP beginning 60 and 14 days, respectively, prior to mating, during mating, gestation and lactation. In the study in which females only were exposed, F1 weanlings were selected from all groups and were given either control diets or the same diets as their mothers for a 7-week post-weaning period.<br>**Results:** In the male fertility study no effect on survival, appearance, behaviour, body wts, hematology and fertility was observed. Organ wts of treated males showed a statistically significantly increased absolute as well as relative liver and kidney wt. at 500 mg/kg bw. Relative kidney wts were also significantly increased in males at 50 and 5 mg/kg bw but these increases were less pronounced, without a dose-relationship. Histopathology of the kidneys did not reveal abnormalities. In addition well-performed 3-month rat studies revealed only at doses ≥350 mg/kg bw increased kidney wts. Therefore the increased kidney wts at 50 and 5 mg/kg bw seen in this male fertility study are considered as biologically insignificant. Reproductive performance, parturition, neonatal viability, growth of newborn, organ wts. and histopathology in weanlings did not reveal abnormalities.<br><br>In the female fertility study no effect on survival, appearance, behaviour, hematology or fertility of treated females was seen. Growth of females was reduced slightly pre-mating, during the entire gestation period and during lactation period at 500 mg/kg bw, statistically significant at week 7, 9 and 11. At 50 mg/kg bw also reductions in weight gain during the entire gestation period were seen, but less pronounced. Organ wts of treated females showed a statistically significantly increased relative kidney wt. at 500 mg/kg bw Histopathology did not reveal abnormalities. Reproductive performance, parturition and neonatal viability did not reveal abnormalities. Pup wt. at birth and growth of pups during entire lactation period was lower at 500 mg/kg bw. Organ wts and histopathology of weanlings did not show abnormalities. During the 7 week post-weaning period also reduced body wts were seen both with and without continuing treatment at all dose-levels, sometimes reaching statistical significance, but without any dose-relationship. Organ wts after 7-week post-weaning period revealed slightly decreased testicular weights in weanlings fed 500 mg/kg bw. After the 7-week post-weaning period histopathology revealed | IRDC, 1984, as summarized in EC, 2003 |

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| testicular lesions in 6/10 weanlings (2 with mild granuloma unilateral, 1 with severe unilateral degradation, 1 with moderate bilateral degeneration, 2 with a trace of bilateral degeneration) fed 500 mg/kg bw. In the group derived from mothers fed 500 mg/kg bw and given control diet for 7 weeks post-weaning, 2/9 weanlings showed testicular lesions (1 with a trace of unilateral degeneration, 1 with severe unilateral degeneration). | |
| **Type/Objective:** Continuous Breeding Protocol to study effects on fertility and reproduction in Swiss (CD-1) mice<br>**Conclusion:** The NOAEL for parental and embryotoxicity is 0.3% in the diet (ca. 420 mg/kg bw).<br>**Method:** Animals received 0, 0.03, 0.3, or1.0% dibutyl phthalate in feed. (ca. 0, 40, 420 and 1,410 mg/kg bw) were administered to groups of 20 M and 20 F animals for a 7-day premating period, after which the animals were grouped as mating pairs and treated during a 98-day mating period. A control group of 40 m and 40 f mice received the basal diet. After the 98-day cohabitation period the pairs were separated and exposed during which period any final litters were delivered and kept for at least 21 days. At the end of the continuous breeding period a 7-day crossover mating trial was performed with Fo animals of control and 1% groups.<br>**Results:** F0 parents showed a significantly decreased growth (males only) and significantly increased liver weights (females only) at 1.0% in the diet. At 1.0% in the diet statistically significant decreases in percentage of fertile pairs, no. of litters/pair, no. of live pups/litter and proportion of pups born alive were seen. Lower dose-levels did not cause these effects. Females and not males were affected as was shown in the crossover mating trial. In this trial between control males and 1.0% females statistically significant decreases in percentage of fertile pairs, no. of live pups/litter, proportion of pups born alive and live pup weight were observed. | Lamb et al, 1987 (Reported by NTP in 1984 and 1995 and summarized in EC, 2003) |
| **Type/Objective:** National Toxicology Program's Reproductive Assessment by Continuous Breeding protocol using Sprague-Dawley rats<br>**Conclusion:** This study showed that DBP is a reproductive/developmental toxicant in Sprague-Dawley rats exposed both as adults and during development; it also indicates that the adverse reproductive/developmental effects of DBP on the second generation were greater than on the first generation and included decreased indices of mating, pregnancy, and fertility, decreased body weight of dams, lower F2 pup weights, decreased epididymal and testicular sperm counts, degenerated seminiferous tubules, and defective epididymides.<br>**Method:** Levels of 0.1, 0.5, and 1.0% DBP in the diet were selected, yielding average daily DBP intakes of 52, 256, and 509 mg/kg for males and 80, 385, and 794 mg/kg for females, respectively. In the study design, Task 2 was a 14-week continuous breeding phase, generating up to five litters per pair. Task 3 consisted of crossover matings between treated and control Fo animals to determine the affected sex, and Task 4 assessed the fertility of the last litter (F1) born during continuous breeding (Task 2).<br>**Results:**<br>Task 2: F0 rats had dose-dependent reductions in total number of live pups per litter in all treated groups by 8-17% and live pup weights in the 0.5% and 1.0% dose groups by < 13%. Postnatal dam weights were significantly reduced (dose-dependent) in all groups.<br><br>Task 3: In crossover mating tests to determine the affected sex, the number of | Wine et al, 1997 (publication of a 1991 NTP study) |

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| offspring was unchanged, but the weights of pups from treated females were significantly decreased and offspring from treated males were unchanged. At necropsy, high-dose F0 females had a 14% reduction in body weight, and both sexes had approximately 10-15% increased kidney and liver to body weight ratios compared to controls. Sperm parameters and estrous cyclicity were not affected.<br><br>Task 4: In the F1 mating trial, indices of mating, pregnancy, and fertility in the 1.0% dose group were all sharply decreased (one live litter was delivered out of 20 cohabited pairs), concomitant with a 13% decrease in dam body weight. Live F2 pup weights were 6-8% lower in all dose groups. F1 necropsy results revealed that epididymal sperm counts and testicular spermatid head counts were significantly decreased in the 1.0% dose group. Histopathologic investigation showed that 8 of 10 F1 males consuming 1.0% DBP had degenerated seminiferous tubules and 5 of 10 had underdeveloped or otherwise defective epididymides. No ovarian or uterine lesions were observed. | |
| **Type/Objective:** Multigenerational study in LE hooded rats<br>**Conclusion:** The lowest dose-level of250 mg/kg bw in this study is a LOAEL based on delayed puberty and urogenital abnormalities, reduced sperm count, reduced fecundity, and other effects in F1 offspring,<br>**Method:** Both male and female animals (10-12 animals/sex/group) of only the P0 generation received orally by gavage 0, 250 or 500 mg DBP/kg bw from weaning, through puberty, young adulthood, mating and lactation. Another group of only males received 1,000 mg/kg bw. When the P0 animals were mated, treated animals were paired with untreated controls. F1 animals were not treated. After puberty F1 animals were selected (16/sex/group) for fertility assessment under continuous mating conditions over 11 breeding cycles.<br>**Results:** In the P0 generation delayed puberty (preputial separation) was seen in males at all dose-levels. DBP treatment did not accelerate the age at vaginal opening or induce persistent vaginal cornification, effects indicative of subchronic estrogen exposure. The P0 generation showed reduced fertility in male and female animals at 500 and 1,000 (males only) mg/kg bw. Infertility in males was related to testicular atrophy and reduced sperm production, while treated females cycled and mated sucessfully, but many treated females (500 mg/kg bw) aborted their litters around midpregnancy.<br><br>In the F1 offspring which were exposed only *in utero* and lactational via dams (data only from F1 animals from dams treated with 0, 250 and 500 mg DBP/kg bw), urogenital malformations/abnormalities including a low incidence of agenesis of the epididymis, hypospadias, ectopic testis, renal agenesis and uterine abnormalities (partial agenesis or lack of implants in one uterine horn) were seen. In addition a few treated animals displayed anophthalmia. Furthermore F1 males from treated mothers exhibited reduced cauda epididymal sperm numbers. The F1 offspring showed reduced fecundity (significantly fewer F2 pups; number pups/litters 179/24, 76/10, and 20/4 for 0, 250 and 500 mg/kg bw, respectively) in similarly treated pairs under continuous breeding conditions. | Gray et al, 1999, as summarized in EC, 2003 |
| **Type/Objective:** Adult male rats in utero exposed to DBP at 250-500 mg/kg/day dose-levels have malformations of the reproductive tract and testicular atrophy. These dose-levels also cause development of multinucleated gonocytes (MNG), inhibit cell proliferation, alter formation of seminiferous tubules, and disrupt contacts between Sertoli and germ cells in the fetal rat testis. The goal of the study was to determine if exposure in utero to low doses of DBP result in these cellular | Kleymenova et al, 2005 |

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| responses in the fetal testis.<br>**Conclusion:**<br>**Method:** Timed-pregnant Sprague-Dawley rats were treated with 0.1, 1, 10, 30, 50, 100, and 500 mg/kg/day DBP by oral gavage on gestation days 12 to 20, and euthanized on day 21. Two hours prior to euthanasia, dams were i.p. injected with 50 mg/kg BrdU. Fetal testes were fixed in situ in modified Davidson's fixative, dissected, and embedded in paraffin. Cellular responses were assessed using H&E- or immunostained with P-cadherin tissue slides.<br>**Results:** The effect of DBP treatment on the size, total cell number, and cordial cross-section number was significant at 50 mg/kg/day dose-level. Although there was a trend indicating that the 50 mg/kg/day dose-level increases the incidence of MNG, statistical significance was achieved only at the 100 mg/kg/day dose-level. Consistent with this trend, fetal rat testes exposed to 30 and 50 mg/kg/day DBP had focal disruption of Sertoli-germ cell contacts as indicated by P-cadherin immunostaining. Our data demonstrate that in the rat, adverse cellular responses in the fetal testis can be detected at lower doses compared to those causing gross pathological changes. The lowest dose that significantly altered size, total cell number, and cordial cross-section number also decreased the concentration of testicular testosterone in the fetal rat testes in utero exposed to DBP. | |
| **Type/Objective:** DBP, DEHP, and BBP were tested in the Hershberger assay.<br>**Conclusion:**<br>**Method:** Immature male Sprague-Dawley rats were castrated at 6 wk of age. Testosterone propionate (TP, 0.4 mg/kg/day) was administered s.c. to castrated male rats and followed by flutamide (1, 5, 10, or 20 mg/kg/day) treatment for 10 days by oral gavage. Similarly, DEHP, DBP, or BBP were also administered by oral gavage at 250, 500, or 1000 mg/kg/day after TP (0.4 mg/kg/day) administration.<br>**Results:** DBP did not affect accessory sex organ weights at any dose. Body weights, combined adrenal glands, and kidney weights were not affected, but liver weights were significantly increased at high dosages in the DEHP, DBP, and BBP treatment groups. | Kang et al, 2005 |
| **Type/Objective:** Effects of long-term oral dosing of DBP to female Long Evans hooded rats on reproductive performance was investigated to determine if DBP might have a significant effect on female reproduction.<br>**Conclusion:** From these two studies, the authors concluded that DBP can cause a negative effect on female fertility at doses of 500 and 1000 mg/kg/day. Also, the F1 generation is more sensitive to phthalate reproductive toxicity than the F0 generation. The authors concluded that the effect of phthalate exposure on female reproduction was previously over shadowed by phthalate effects on male reproduction because an effect on pregnancy is not seen with shorter term studies. In addition, in standard testing treated females are mated with treated males. As a result of no obvious changes in females, it may have been assumed that infertility was due to the altered male reproductive tract development induced by phthalate exposure.<br><br>**Method:** In the first of two studies, 21-day old females were given an oral dose of DBP [0 (n=12) or 500 mg/kg/day (n=8)] for the duration of the study (gd 13 of third pregnancy). Females were examined for vaginal opening daily, and then estrous cyclicity was evaluated daily by vaginal smears. At day 83, each female rat was mated for 14 days to treated male rats. Litters were then counted and weighed at birth and postnatal day 15, when they were euthanized. The same | Gray et al, 2006 |

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| female rats were then mated to untreated male rats, after a 30 day recovery period. Litters were euthanized at weaning (day 21). The same 12 female rats were mated for a third time, to untreated male rats. At gd 13, the 12 female rats were euthanized and necropsied, fetuses counted, and serum collected for progesterone analysis.<br><br>In the 2nd study, 24 day old female rats (n=12-13) were orally dosed with 0, 250, 500, or 1000 mg/kg/day DBP five days a week until day 110, and then they were dosed 7 days a week until euthanization during the second pregnancy. On the first day of proestrus, female rats were mated for 24 hours to untreated male rats. Pups were counted and weighed at days 1, 5, and 15 before euthanization. The female rats were remated to untreated male rats for 24 hours. At gd 13, rats were euthanized by CO2, serum was collected for hormone analysis, organ weights evaluated, fetuses counted, and stimulated ovary hormone evaluated *ex vivo*.<br><br>**Results:** In the 1st study, DBP did not affect maturation, estrous cyclicity, or percentage of females mating or pregnant. However, the results did indicate that there was a significant decrease in the number of live pups delivered by treated females in both pregnancy 1 and 2 ($p<0.05$). The presence of blood in the vaginal lavages of some of the females suggested mid-pregnancy losses. At mid-pregnancy necropsies during the third pregnancy, researchers found no reduction of implantations in the DBP treated females, but did view a decrease in the percentage of viable fetuses. This is consistent with the reduced litter sizes of the first two pregnancies.<br><br>Chronic exposure to DBP in the 2nd study did not affect female rat growth or ability to mate. Results did show that 42% and 8% of treated females receiving 500 and 1000 mg/kg/day DBP were fertile when compared to 92% for untreated female rats. Litter sizes were also significantly reduced at these doses when compared to control animals ($p<0.01$). A large number of the pregnant females in the 500 and 1000 mg/kg/day treated group did not produce live pups and presented consistent pregnancylike vaginal lavages with detectable blood at mid-pregnancy. During necropsy at gd 13, uterine weights ($p<0.05$, $p<0.05$) and the number of live fetuses ($p<0.01$, $p<0.01$) and total number of fetuses ($p<0.01$, $p<0.05$) were significantly reduced at the two highest doses of DBP (500 and 1000 mg/kg/d). The ovaries showed visible hemorrhagic corpora lutea, and the serum progesterone was significantly reduced at 1000 mg/kg/day (to 25%, $p>0.1$). When ovaries of females with live fetuses were stimulated *ex vivo*, the 500 and 1000 mg/kg/day dose groups had significantly reduced ($p<0.001$) progesterone production and increased estradiol production. Ovaries from female rats with no live fetuses had low progesterone production similar to those seen in non-pregnant rats. | |
| **Type/Objective:** No information is available on the potential adverse effects of DBP during fetal development in higher primates. In primates, androgens from the fetal adrenal are responsible for the ability of the placenta to produce the estrogens of pregnancy. The purpose of this study was to determine if maternal DBP exposure during early pregnancy in female cynomolgus macaques would result in lower maternal estrogen excretion, indicating that the fetal adrenal is a target for DBP.<br>**Conclusion:** These data support the concept that DBP treatment (500 mg/kg bw daily orally) for 6 weeks during the time of fetal adrenal formation can suppress | Gee et al, 2007 |

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| fetal adrenal androgen production in higher primates. Furthermore, the data provide evidence that the human fetal adrenal may be a target for DBP toxicity.<br>**Method:** Four pregnant female cynomolgus macaques (M. fascicularis) were treated daily with DBP (500 mg/kg BW in corn oil) administered orally via naso-gastric intubation. Treatments were initiated between gestational days (GD) 25 - 28 and continued for a total of 6 weeks. Two pregnant animals served as untreated controls. All animals were time mated and the day of ovulation was determined by analysis of daily urinary samples for estrone conjugates (E1C) and ß-FSH. The day of ovulation was assigned GD 0. Pregnancy was determined by serum macaque chorionic gonadotropin (mCG) levels and confirmed by ultrasound prior to the first treatment.<br>**Results:** Preliminary findings indicate that the normal increase of estrogen production during early pregnancy is reduced by DBP exposure. Urine samples collected from GD 37 - 51 were analyzed for urinary estrogen metabolites (E1C) and indexed by creatinine (Cr) to account for variations in urine concentration. The mean E1C slope determined using linear regression was 7.6 ng/mg Cr/day in the DBP treated group and 15.5 ng/mg Cr/day for controls. This two-fold difference between the groups was not statistically significant ($P>0.05$) due to daily variations in E1C. When the mean area under the curve (AUC, calculated by the trapezoidal rule) was compared across the two groups, the difference was found to be statistically significant ($P<0.05$). The mean E1C AUC was 1502 ng•day/mg Cr for DBP treated animals and 2912 ng•day/mg Cr for the controls. | |
| **Type/Objective:** A study was conducted to evaluate DBP's effect on reproductive function of Wistar rats.<br>**Conclusion:** Evidence indicates that DBP exposure causes dose-dependent testicular toxicity and has the potential to induce adverse effect.<br>**Method:** DBP was given orally at a dose of 500, 1000 and 1500 mg/kg bw for 7 days. Histological and fertility parameters were assessed.<br>**Results:** Significant reduction in seminiferous tubule diameter, Leydig cell nuclear diameter (except at dose 500 mg), number of primary spermatocytes, secondary spermatocytes and spermatids were observed. Caudal sperm density and viability reduced significantly. Decrease in serum testosterone was also observed. | Nairet al, 2008 |
| **Type/Objective:** Reproductive effects of low-dose DBP, including expression of proteins, was investigated.<br>**Conclusion:** High doses of DBP led to testicular toxicity, and low doses of DBP led to changes in the expression of proteins involved in spermatogenesis as well as changes in the number and function of Sertoli and Leydig cells, although no obvious morphological changes appeared.<br>**Method:** Pubertal male Sprague-Dawley rats were orally administered DBP at a wide range of doses (0.1, 1.0, 10, 100 and 500 mg $kg^{-1}$ $day^{-1}$) for 30 days. The selected end points included reproductive organ weights, testicular histopathology and serum hormonal levels. Additionally, proteomic analysis was performed to identify proteins that are differentially expressed as a result of exposure to DBP at low doses (0.1, 1.0 and 10 mg $kg^{-1}$ $day^{-1}$).<br>**Results:** Toxic effects were observed in the high-dose groups, including anomalous development of testes and epididymides, severe atrophy of seminiferous tubules, loss of spermatogenesis and abnormal levels of serum hormones. Treatment with low doses of DBP seemed to exert a 'stimulative effect' on the serum hormones. Proteomics analysis of rat testes showed 20 differentially expressed proteins. Among these proteins, alterations in the expression of | Bao et al, 2011 |

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| HnRNPA2/B1, vimentin and superoxide dismutase 1 (SOD1) were further confirmed by Western blot and immunohistochemistry. | |
| **Type/Objective:** The aim of this study was to assess the impact of di(n-butyl) phthalate (DBP) on the rat's prepubertal testis.<br>**Conclusion:** Exposure of a rat to DBP in doses 100 or 1,000-fold higher than a Tolerable Daily Intake for humans had no effect on its testicular development.<br>**Method:** Male Wistar rats were given daily subcutaneous injections with DBP (20 or 200 μg) or a vehicle from the 5th to the 15th postnatal day (pd). On the 16(th) pd, the rats were euthanized, and the testes were dissected, weighed, and paraffin embedded. The blood was collected to determine the serum levels of testosterone (T), estradiol (E) and FSH. The following parameters were assessed in the testis sections: diameter and length of seminiferous tubules (st), numbers of spermatogonia A + intermediate + B (A/In/B), preleptotene spermatocytes (PL), leptotene + zygotene + pachytene spermatocytes (L/Z/PA) and Sertoli cells per testis, percentage of st containing gonocytes or pachytene spermatocytes or lumen. An estrogenicity in vitro test was performed by means of a transgenic yeast strain expressing human estrogen receptor alpha.<br>**Results:** At both doses, DBP had no influence on testis and seminal vesicle weight, st diameter and length, number of germ and Sertoli cells per testis, percentage of st containing gonocytes or pachytene spermatocytes or lumen. DBP did not change E, T or FSH serum levels. The in vitro yeast screen showed that DBP was a weak estrogenic compound, approximately six to seven orders of magnitude less potent than 17β-estradiol. | Filipiak et al, 2011 |
| **Type/Objective:** A multigenerational study was performed in mice to investigate the effects of paternal DBP exposure pre- and postnatally on F1 generation offspring, and prenatally on F2 generation offspring.<br>**Conclusion:** Paternal DBP exposure may disturb the sex ratio of the offspring, delay female sexual maturation, and deteriorate the sperm quality of F1 generation males.<br>**Method:** Male mice were exposed to either 500 mg/kg or 2 000 mg/kg of DBP for 8 weeks, and mated with non-exposed females. Three-quarters of the females were sacrificed a day prior to parturition, and examined for the number of living and dead implantations, and incidence of gross malformations. Pups from the remaining females were assessed for developmental markers, growth parameters, as well as sperm quantity and quality.<br>**Results:** There were no changes in the fertility of parents and in intrauterine development of the offspring. Pups of DBP-exposed males demonstrated growth-retardation. Following paternal exposure to 500 mg/kg bw of DBP, there were almost twice the number of males than females born in the F1 generation. F1 generation females had a 2.5-day delay in vaginal opening. Paternal exposure to 2 000 mg/kg bw of DBP increased the incidence of sperm head malformations in F1 generation males; however, there were no changes in the fertility and viability of foetuses in the F2 generation. | Dobrzyriska et al, 2011 |
| **Type/Objective:** Epidemiological data indicating increased incidence of testicular dysgenesis in boys exposed to phthalates in utero are reinforced by studies demonstrating that phthalates impair fetal rodent testis development. Because humans are exposed to phthalates continuously from gestation through adulthood, it is imperative to understand what threat phthalates pose at other life stages.<br>**Conclusion:** These data demonstrate the acute sensitivity of the prepubertal mouse testis to DBP at doses 50- to 500-fold lower than those used in rat and identify the upregulation of inhibin as a potential mechanism of DBP action. | Moody et al, 2013 |

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| **Method:** To determine the impact during prepuberty, we assessed the consequences of oral administration of 1 to 500 mg di-n-butyl phthalate (DBP)/kg/d in corn oil to wild-type (C57BL/6J) male mice from 4 to 14 days of age.<br>**Results:** Dose-dependent effects on testis growth correlated with reduced Sertoli cell proliferation. Histological and immunohistochemical analyses identified delayed spermatogenesis and impaired Sertoli cell maturation after exposure to 10 to 500 mg DBP/kg/d. Interference with the hypothalamic-pituitary-gonadal axis was indicated in mice fed 500 mg DBP/kg/d, which had elevated circulating inhibin but no change in serum FSH. Increased immunohistochemical staining for inhibin-α was apparent at doses of 10 to 500 mg DBP/kg/d. Serum testosterone and testicular androgen activity were lower in the 500 mg DBP/kg/d group; however, reduced anogenital distance in all DBP-treated mice suggested impaired androgen action at earlier time points. Long-term effects were evident, with smaller anogenital distance and indications of disrupted spermatogenesis in adult mice exposed prepubertally to doses from 1 mg DBP/kg/d. | |
| **Type/Objective:** This study aimed to observe the possible protective effects of resveratrol (RSV) against damage induced by DBP on the ductus epididymis and deferens in rats.<br>**Conclusion:** DBP administration caused structural degeneration in the epididymis and deferens, parallel to dose evaluation and RSV can reverse these changes with its protective effects.<br>**Method:** Six groups of rats were used in the experiment: Group 1: Control group; Group 2: Solvent (carboxymethylcellulose (CMC), 10 ml/kg); Group 3: 500 mg/kg/day DBP; Group 4: 500 mg/kg/day DBP+20 mg/kg/day RSV; Group 5: 1000 mg/kg/day DBP; Group 6: 1000 mg/kg/day DBP + 20 mg/kg/day RSV. Groups were treated by gavage for 30 days. Immunohistochemical, electronmicroscopic and histomorphometric examinations were carried out in the epididymis and deferens.<br>**Results:** In the ductus epididymis and deferens mitochondrial crystolysis, exfoliation of the stereocilia and openings in lateral surface increased with DBP dosage, but these structures were recovered with RSV. DBP reduced the epithelial height of epididymis and vas deferens. Lumen dilatation was observed in both tissues. These disorders may lead to dysfunction of epithelial absorption. In the TUNEL examinations in both tissues, there were no apoptotic cells or apoptotic bodies. | Sahin et al, 2014 |
| **Type/Objective:** To investigate the effects of DBP given in diet on reproductive endpoints in male quails.<br>**Conclusion:** Data were consistent with previous reports showing that DBP modulates Leydig cell steroidogenesis in several species, with a potential negative effect on reproduction in those avian species that are vulnerable to endocrine disrupting chemicals.<br>**Method:** Authors investigated the effects of 30-day dietary (pre-pubertal) exposure to different doses (0 (control), 1, 10, 50, 200 and 400 mg/kg bodyweight/day) of DBP on Leydig cells of adult male Japanese quails by quantifying the transcript levels for P450 side-chain cleavage (p450scc), P450c17 (CYP17), and 3β- and 17β-hydroxysteroid dehydrogenase (hsd) using quantitative (real-time) polymerase chain reaction (qRT-PCR). In addition, the plasma testosterone levels were analysed using radioimmunoassay (RIA) and testis was examined for evidence of gross pathology and histopathology.<br>**Results:** Data showed that pre-pubertal exposure to DBP produced alterations in | Bello et al, 2014 |

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| testicular architecture as evident by poorly developed or mis-shaped testis, and altered spermatogenesis due to tubular degeneration and atrophy of seminiferous tubules especially in the high DBP dose (200 and 400 mg/kg) treated groups. In addition, DBP altered several key enzymes involved in testicular steroidogenesis pathways in an apparent dose-dependent manner. For example, biphasic effects of DBP were observed for P450scc and 3β-hsd mRNA, that were generally increasing at low dose 10 mg/kg, and thereafter, an apparent dose-dependent decrease between 50 and 400mg/kg. The steroidogenic acute regulatory (StAR) protein was at the lowest detectable limits and therefore not quantifiable. These effects did not parallel the non-significant changes observed for plasma testosterone levels. | |
| **Type/Objective:** The estrogenic chemical nonylphenol (NP) and the antiandrogenic agent DBP are regarded as widespread environmental endocrine disruptors (EDCs) which at high doses in some species of laboratory animals, such as mice and rats, have adverse effects on male reproduction and development. Their combined effects warrant clarification.<br>**Conclusion:** The potential of Bliss Independence model for the prediction of interactions between estrogenic and antiandrogenic agents was demonstrated.<br>**Method:** Authors attempted to determine the mixture effects of NP and DBP on the testicular Sertoli cells and reproductive endocrine hormones in serum in male rats based on quantitative data analysis by a mathematical model. In the in vitro experiment, monobutyl phthalate (MBP), the active metabolite of DBP, was used instead of DBP. Sertoli cells were isolated from 9-day-old Sprague-Dawley rats followed by treatment with NP and MBP, singly or combined. Cell viability, apoptosis, necrosis, membrane integrity and inhibin-B concentration were tested. In the in vivo experiment, rats were gavaged on postnatal days 23-35 with a single or combined NP and DBP treatment. Serum reproductive hormone levels were recorded. Next, Bliss Independence model was employed to analyze the quantitative data obtained from the in vitro and in vivo investigation.<br>**Results:** Antagonism was identified as the mixture effects of NP and DBP (MBP). | Hu et al, 2014 |
| **Type/Objective:** Fetal growth and fetal androgen exposure can also predetermine testosterone levels in men, although how is unknown, because the adult Leydig cells (ALCs) that produce testosterone do not differentiate until puberty. To explain this conundrum, we hypothesized that stem cells for ALCs must be present in the fetal testis and might be susceptible to programming by fetal androgen exposure during masculinization.<br>**Conclusion:** DBP was used to reduce intratesticular testosterone in rats, probably by reduced testicular steroidogenic acute regulatory protein expression, which is associated with increased histone methylation (H3K27me3) in the proximal promoter. Subsequent findings included reduced adult Ledig cell (ALC) stem cell number by ∼40% at birth to adulthood and induced compensated ALC failure (low/normal testosterone and elevated luteinizing hormone).<br>**Method:** To address this hypothesis, we used ALC ablation/regeneration to identify that, in rats, ALCs derive from stem/progenitor cells that express chicken ovalbumin upstream promoter transcription factor II. These stem cells are abundant in the fetal testis of humans and rodents, and lineage tracing in mice shows that they develop into ALCs. The stem cells also express androgen receptors (ARs).<br>**Results:** Reduction in fetal androgen action through AR KO in mice or dibutyl phthalate (DBP) -induced reduction in intratesticular testosterone in rats, reduced | Kilcoyne et al, 2014 |

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| ALC stem cell number by ~40% at birth to adulthood, and induced compensated ALC failure (low/normal testosterone and elevated luteinizing hormone). In DBP-exposed males, this failure was probably explained by reduced testicular steroidogenic acute regulatory protein expression, which is associated with increased histone methylation (H3K27me3) in the proximal promoter. Accordingly, ALCs and ALC stem cells immunoexpressed increased H3K27me3, a change that was also evident in ALC stem cells in fetal testes. | |
| **Type/Objective:** To further explore the potential testicular toxicity of DBP in adult rats and to elucidate the underlying mechanisms.<br>**Conclusion:** These results indicated that oxidative stress and subsequent decrease in testosterone secretion were the potential underlying mechanism of DBP-induced testicular toxicity.<br>**Method:** Adult male albino rats were treated orally with DBP at doses of 0, 200, 400, or 600 mg/kg/day for 15 consecutive days. Testicular weight, sperm count, and motility were significantly decreased.<br>**Results:** Treatment with DBP decreased serum follicle-stimulating hormone and testosterone levels and testicular lactate dehydrogenase activity. DBP treatment also decreased serum total antioxidant capacity and the activities of the testicular antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione reductase. Further, DBP treatment provoked degeneration with absence of spermatogenesis and sperms and necrosis in some of seminiferous tubules. | Aly et al, 2015 |
| **Effects on reproductive organs with postnatal dosing, but without mating** | |
| **Type/Objective:** To investigate species differences after oral dosing with DBP<br>**Conclusion:** Species differ widely in their sensitivity to the testicular toxicity of **phthalate** esters.<br>**Method:** Oral administration<br>**Results:** Oral administration of DBP produced uniformly severe seminiferous tubular atrophy in rats and guinea pigs but caused only focal atrophy in mice. Hamsters showed no testicular changes with DBP and only minor changes in response to DEHP and di-n-pentylphthalate (DPP). The rate of intestinal monohydrolysis of DEHP was slower in hamsters than in rats and this may be important, as mono-(2-ethylhexyl)**phthalate** (MEHP) did cause focal seminiferous tubular atrophy in hamsters. However, MBP had no such effect. The decrease in testicular zinc concentration and enhancement of urinary zinc excretion produced in rats by DEHP and DPP was not observed in hamsters. | Gray et al, 1982 |
| **Type/Objective:** Testicular effects of short-term dosing with DBP in rats was investigated.<br>**Conclusion:** LOAEL was 2400 mg/kg/day (only dose tested).<br>**Method:** Male Wistar rats (28/group) received 0 or 2400 mg/kg-day by gavage for 7 days. Rats were killed at various times during and up to 96 hours after treatment ended. The testes were removed and weighed. The left testis was examined for histopathological changes. The right testis was homogenized and the homogenates were assayed for phospholipids, triacylglycerols, cholesterol, glucose, fructose, galactose, inositol, aldose reductase, zinc, iron, and sorbitol dehydrogenase. Blood samples were collected and assayed for fructose, glucose, and inositol.<br>**Results:** DBP caused slight sloughing of germ cells in the seminiferous tubules after 24 hours. Severe sloughing was observed at 48 hours. The germ cells almost completely disappeared from the germinal epithelium after 5 and 7 days with only | Fukuoka et al, 1989 |

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| Sertoli cells left in the germinal epithelium. Testicular fructose and glucose concentrations were decreased after 24 hours ($p< 0.05$) and not detectable at 48 hours and longer. Testicular zinc and iron concentrations were decreased and inositol and cholesterol were increased after 48 hours. Testicular triacylglycerols, cholesterol, and phospholipids containing choline and ethanolamine residues were decreased at later times. Sorbitol dehydrogenase activity was significantly elevated after 24 hours ($p< 0.05$) and significantly decreased after 5 and 7 days ($p< 0.05$). Blood fructose, glucose, and inositol concentrations were not significantly affected at 24 hours. The authors concluded that dibutyl phthalate causes sloughing of germ cells from seminiferous tubules leaving only Sertoli cells. The decreases in glucose and fructose concentration suggest that dibutyl phthalate may disturb an interaction between Sertoli cells and germ cells. | |
| **Type/Objective:** To evaluate effects of DBP on testis during early life<br>**Conclusion:** The alterations in activity of testicular cell specific enzymes suggest that DBP exposure during early life could affect the testicular functions. The LOAEL was 250 mg/kg/day, the lowest dose tested.<br>**Method:** DBP was administered to young male rats by gavage at the doses of 250, 500 and 1,000 mg/kg body weight/day for 15 days.<br>**Results:** A significant decrease in testes weight was observed at 500 and 1,000 mg/kg doses of DBP. Histopathological examination revealed marked degeneration of seminiferous tubules. The activities of testicular enzymes associated with postmeiotic spermatogenic cells, such as sorbitol dehydrogenase and acid phosphatase, were decreased significantly, while that of lactate dehydrogenase was significantly increased, coincident with degeneration of spermatogenic cells. The activities of enzymes associated with premeiotic spermatogenic cells, Sertoli cells or interstitial cells, beta-glucuronidase, gamma-glutamyl transpeptidase and glucose-6-phosphate dehydrogenase were significantly increased. | Srivastava et al, 1990 |
| **Type/Objective:** The aim of this study was to identify the DBP-induced differentially regulated genes (DEGs) in the testes of male rats using a novel annealing control primer (ACP) system.<br>**Conclusion:** These results suggest that the spermatogenesis-related genes identified in this study will provide insights into the molecular mechanisms of DBP on the testicular development and dysgenesis.<br>**Species/strain:**<br>**Method:** Sprague-Dawley male rats (4 weeks of age) were treated with DBP (250, 500, or 750 mg/kg/day) by oral gavage for 30 days. The total RNA was isolated from the rat testes, and the differential gene expression levels were determined. Using this technique, total of 59 DEGs mRNA fragments were observed in the testes treated with DBP 750 mg/kg/day compared with the vehicle control.<br>**Results:** Of 59 genes, 31 genes were altered significantly after exposing the rats to high dose of DBP (750 mg/kg/day), and their sequences were cloned. Based on the Basic Local Alignment Search Tool (BLAST), 4 expressed sequence tags (EST) and 27 cloned genes (Insl3, pgrp, H1SHR etc.,) were identified. A further examination of 3 genes involved in spermatogenesis and steroidogenesis in the testis from this profile was carried out using reverse transcription PCR (RT-PCR). Significant differences in the expression levels of these genes (LDH, lactate dehydrogenase; spag4, a spermatid specific gene and BPR, benzodiazepine receptor) were observed between the DBP-treated and control groups. | Ahn et al, 2006 |

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| **Type/Objective:** The present study focused on investigating whether the inhibitory effect of DBP on testosterone (T) biosynthesis was mediated by the glucocorticoid (GC) pathway in prepubertal male rats and T production after the exposure to DBP ceased.<br>**Conclusion:** These data suggest that DBP inhibits testosterone production through a GC-mediated pathway in prepubertal male rats, and after exposure to DBP ceases, testosterone biosynthesis returns.<br>**Method:** Prepubertal male rats were administered DBP in corn oil orally at 0, 250, 500, 1000, and 2000 mg/kg daily for 30 days. Serum T and GC were measured by radioimmunoassay and enzyme-linked immunosorbent assay, respectively. The responses, including glucocorticoid receptor (GR), type I 11beta-hydroxysteroid dehydrogenase (11beta-HSD1), and steroidogenesis acute regulatory protein (StAR) in the testes tissues, were determined by Western blotting and reverse transcriptase PCR.<br>**Results:** DBP exposure resulted in testicular toxicity, such as seminiferous tubule degeneration and a decrease in the number of spermatogenic cells. T was decreased and GC was increased in a DBP concentration-dependent manner in the exposure group. The expression of GR and 11beta-HSD1 was significantly increased, with an associated decrease in expression of StAR. Neither the expression of the GR nor 11beta-HSD1 and StAR were statistically significantly different in the postexposure group compared with the control. However, the weight and morphology of the testes did not recover in the postexposure group. | Xiao-feng et al, 2009 |

**BACKGROUND INFORMATION FOR TABLE 35B: HUMAN DATA ON ADVERSE EFFECTS ON SEXUAL FUNCTION AND FERTILITY**

| Summary of Study | Reference |
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| **Type/Objective:** The present study explored whether environmental levels of **phthalates** are associated with altered semen quality in humans.<br>**Conclusion:** There were dose-response relations for monobutyl **phthalate** and monobenzyl **phthalate** with one or more semen parameters, and suggestive evidence for monomethyl **phthalate** with sperm morphology.<br>**Method:** Authors recruited 168 men who were part of subfertile couples and who presented to the Massachusetts General Hospital andrology laboratory for semen analysis between January 2000 and April 2001. This was a cross sectional study in which semen and urine samples were collected from each subject on the same day as part of an infertility work-up. Semen parameters were categorized based on 1999 World Health Organization (WHO) reference values for sperm concentration (<20 million/ml) and motility (<50% motile), as well as Tygerberg strict criteria for morphology (<4% normal). The comparison group was men ( n = 77) for whom these semen parameters were all above the reference values. The concentration of eight phthalate monoesters was measured in a single spot urine sample with high-performance liquid chromatography and tandem mass spectrometry. Exposure to chemicals other than phthalate esters was not evaluated. Specific gravity-adjusted phthalate monoester levels were subdivided into tertiles (0!11.64, 12.24!20.13, and 20.16!433.93 ng/mL).<br>**Results:** There was a statistically significant relationship between tertiles of urinary monobutyl phthalate and decreased sperm motility (odds ratio per tertile = 1.0, 1.8, 3.0; p-value for trend 0.02). There was a suggestive relationship between tertiles of urinary monobutyl phthalate and decreased sperm concentration (odds ratio per tertile = 1.0, 1.4, 3.3; p-value for trend 0.07). There was also a statistically significant relationship for monobenzyl phthalate with sperm concentration. | Duty et al, 2003 |
| **Type/Objective:** Survey of urine, sperm, and semen endpoints and phthalates during military medical examinations.<br>**Conclusion:** No association between MBP and reproductive endpoints was found.<br>**Method:** Authors collected urine, sperm, and semen samples from men undergoing military medical examinations. Sperm concentration, motility, and integrity; semen volume; epididymal and prostate function; and serum reproductive hormones were evaluated.<br>**Results:** For those whose urine tested positive for monobutyl phthalate (MBP), a metabolite of DBP, no association between DBP exposure and the reproductive endpoints were found. | Jönsson et al. (2005), as summarized in CPSC, 2010 |
| **Type/Objective:** DBP concentration was measured and semen quality was evaluated in samples from men at reproductive institute in Shanghai.<br>**Conclusion:** A possible relation between DBP concentration and sperm motility was seen. | Zhang et al, 2006, as summarized in CPSC, 2010 |

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| **Method:** In Shanghai, semen from men ages 23 to 48 was collected from the Shanghai Institute of Planned Parenthood Research. All men were out patients, but it is unclear if any of the men had a previous reproductive history. DBP concentration was measured and semen quality was evaluated.<br>**Results:** Authors reported no correlation between DBP concentration in the semen and sperm concentration or viability. The authors noted a positive correlation between liquefied time of semen (the amount of time it takes for the semen to become liquid at room temperature) and DBP concentration, and a negative correlation coefficient associated with semen quality and DBP concentration. The author stated that the negative correlation coefficient suggests that phthalates could affect sperm motility. | |
| **Type/Objective:** Previously, the authors reported dose-response associations of decreased semen quality with urinary concentrations of monobutyl **phthalate** (MBP) and monobenzyl (MBzP) **phthalate**, metabolites of parent phthalate diesters The present study extends our work in a larger sample of men and includes measurements of di(2-ethylhexyl) **phthalate** (DEHP) oxidative metabolites.<br>**Conclusion:** This study in 463 men from subfertile couples confirms previous results on the relationship of altered semen quality (low sperm concentration) with exposure to MBP at general population levels.<br>**Method:** Between January 2000 and May 2004, we recruited 463 male partners of subfertile couples who presented for semen analysis to the Massachusetts General Hospital. Semen parameters were dichotomized based on World Health Organization reference values for sperm concentration (<20 million/mL) and motility (<50% motile) and the Tygerberg Kruger Strict criteria for morphology (<4% normal). The comparison group was men with all 3 semen parameters above the reference values. In a single spot urine sample from each man, **phthalate** metabolites were measured using solid-phase extraction coupled to high-performance liquid chromatography isotope-dilution tandem mass spectrometry.<br>**Results:** There were dose-response relationships of MBP with low sperm concentration (odds ratio per quartile adjusted for age, abstinence time, and smoking status = 1.00, 3.1, 2.5, 3.3; P for trend = 0.04) and motility (1.0, 1.5, 1.5, 1.8; P for trend = 0.04). There was suggestive evidence of an association between the highest MBzP quartile and low sperm concentration (1.00, 1.1, 1.1, 1.9; P for trend = 0.13). There were no relationships of monoethyl **phthalate**, monomethyl **phthalate**, and the DEHP metabolites with these semen parameters. | Hauser et al, 2006 |
| **Type/Objective:** The the effects of DBP on female reproduction were investigated.<br>**Conclusion:** Higher serum DBP concentrations may be associated with increased endometriosis in women.<br>**Method:** Blood samples were collected from infertile women with endometriosis and those without endometriosis, but having other causes of infertility (Reddy, 2006). In addition, blood samples were collected from fertile women with no history of gynecological disorders. DBP was measured by gas chromatography.<br>**Results:** There was a significant increase of DBP in the blood of infertile women with endometriosis compared to infertile women without endometriosis and fertile women ($p<0.05$). There was no significant difference in phthalate concentration between the infertile women without endometriosis and the fertile women. | Reddy et al, 2006, as summarized in CPSC, 2010 |
| **Type/Objective:** To assess the associations between hazard index (HI) of cumulative DBP and DEHP exposures and serum concentrations of free | Pan et al, 2011 |

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| testosterone (fT), estradiol, luteinizing hormone (LH) and follicle-stimulating hormone (FSH).<br>**Conclusion:** Both T production and hypothalamo-pituitary-testis (HPT) axis function were damaged in workers with high HI of phthalate exposures. HPT feedback function was activated in workers with both high and low HI, and plays an important role in preventing fT level from further decreasing with a rise in HI.<br>**Method:** We used restricted cubic spline function to characterize the dose-response curves between the HI values and reproductive hormones for 74 male workers occupationally exposed to high levels of DBP and DEHP, and 63 male construction workers as comparison group matched for age and smoking status.<br>**Results:** The median of HI value was 5.30 for exposed workers, 53.0-fold that of unexposed workers (0.10). 89.2% of exposed workers and 1.6% of unexposed workers have HI over 1.00. We observed a borderline significantly negative association between HI and fT in exposed workers (r=-0.195, p=0.096), but not in unexposed workers. The exposed workers showed inverted long-tailed J-shaped fT and FSH curves, and small changes in the LH curve, whereas unexposed workers had inverted and flattened-S-shaped fT and mirror-S-shaped LH and FSH curves. | |
| **Type/Objective:** This study was done to help show a correlation between epidemiological studies with phthalates and in vitro data for the effect of phthalate esters.<br>**Conclusion:** Sperm from men at an infertility clinic were exposed for 0.5 to 96 hr to phthalates. Motility of sperm was decreased under in vitro conditions at the maximum range of in vivo measured levels and 5- or 10-folds higher to that found in human semen samples.<br>**Method:** Healthy human males, in the age group 21 to 40 years, visiting Chhatrapati Sahuji Maharaj Medical University (CSMMU), Lucknow, as part of infertility investigation, were recruited as volunteers. Semen analysis was performed according to the WHO guidelines. Phthalate esters were analyzed by high-performance liquid chromatography (HPLC) and cell viability by MTT assay. In the in vitro studies, sperms were exposed to highest concentration in semen samples (5-10 times higher) for a period ranging between 30 min and 96 hours.<br>**Results:** An inverse relationship with sperm motility in epidemiological studies was concurrent by significant dose-and time-dependent decrease in the sperm motility under in vitro environment after 12-hour exposure. Cytotoxicity was observed only with the highest concentration after 96 hours of exposure. There are a significant correlation between phthalate ester diethylhexyl phthalate, di-n-butyl phthalate (DEHP and DBP) and sperm motility both in vitro and in vivo conditions. Additionally, in vitro experiments conducted not only adjunct to the existing in vivo data but also specify the effect of specific toxicants (DEHP and DBP) on sperm motility and viability. | Pant et al, 2011 |
| **Type/Objective:** To evaluate the influence of phthalates on human luteal cell function.<br>**Conclusion:** The results show the ability of phthalates to affect luteal steroidogenesis as well as the balance between luteotrophic and luteolytic factors suggesting an interference of phthalates in human luteal function. These data may contribute to clarify the classically known impaired reproductive health observed after phthalates exposure.<br>**Method:** Human luteal cells were isolated from corpora lutea for primary cultures from 23 normally menstruating patients in the midluteal phase. Authors | Romani et al, 2014 |

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| investigated the effect of di(2-ethylhexyl)phthalate (DEHP), di-n-butyl phthalate (DBP), and butyl benzyl phthalate (BBP) on basal and hCG-induced progesterone (P4) release, as well as DEHP effect on the balance between prostaglandin (PG) E2, vascular endothelial growth factor (VEGF)-luteotrophic factors, and the luteolitic PGF2α in isolated human steroidogenc cells. Main endpoints were progesterone (P4) and prostaglandin release assayed by enzyme immunoassay, vascular endothelial growth factor (VEGF) secretion by enzyme-linked immunosorbent assay (ELISA), and VEGF mRNA expression by real-time polymerase chain reaction. Influence of phthalates on VEGF expression has been also evaluated.<br>**Results:** DEHP, DBP, and BBP were able to reduce both basal and hCG-stimulated P4 as well as PGE2 release. PGF2α release was reduced after DEHP incubation. VEGF protein release was decreased by the incubation with the tested phthalates. VEGF mRNA expression was not affected by DEHP, DBP, and BBP. As expected, both hCG and cobalt chloride were able to induce P4 release and VEGF release and mRNA expression in human luteal cells respectively. | |
| **Type/Objective:** There is evidence of declining trends in testosterone (T) levels among men in recent decades, as well as trends in related conditions at multiple life stages and in both sexes. This study was performed to explore relationships between urinary concentrations of 13 phthalate metabolites and serum total T levels among men, women, and children when adjusting for important confounders and stratifying by sex and age (6-12, 12-20, 20-40, 40-60, and 60-80 y) using a cross-sectional study design.<br>**Conclusion:** Suggestive relationships were found between phthalates and reduced testosterone, including an association in men who were 40-60 years old.<br>**Method:** Main endpoint: Serum total T measured by isotope dilution-liquid chromatography-tandem mass spectrometry.<br>**Results:** Multiple phthalates were associated with significantly reduced T in both sexes and in differing age groups. In females, the strongest and most consistent inverse relationships were found among women ages 40-60 years. In boys 6-12 years old, an interquartile range increase in metabolites of di-2-ethylhexyl phthalate was associated with a 29% (95% confidence interval, 6, 47) reduction in T. In adult men, the only significant or suggestive inverse associations between phthalates (metabolites of di-2-ethylhexyl phthalate and dibutyl phthalate) and T were observed among men ages 40-60 years. | Meeker and Ferguson, 2014 |
| **Type/Objective:** To examine associations between phthalate metabolite urinary concentrations during early pregnancy and blood glucose levels obtained at the time of screening for gestational diabetes mellitus (GDM).<br>**Conclusion:** Women in this study with the highest urinary concentrations of MiBP and MBzP had lower blood glucose levels. Because maternal glucose levels increase during pregnancy to provide adequate nutrition for fetal growth and development, these findings may have implications for fetal health.<br>**Method:** Upon initiation of prenatal care, women with a mean gestational age of 12.8 weeks were recruited for a study of environmental chemical exposures (n=110) and provided a spot urinary specimen. Blood glucose concentrations (mg/dl) were obtained from the electronic medical record for those patients who did not experience a pregnancy loss and did not transfer care to another facility prior to glucose screening (n=72). Urinary concentrations of nine phthalate metabolites and creatinine were measured at the US Centers for Disease Control and Prevention. Associations between tertiles of phthalate metabolites concentrations and blood glucose levels were estimated using linear regression. | Robledo et al, 2015 |

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| **Results:** Compared to pregnant women in the lowest concentration tertile, women with the highest urinary concentrations (≥3rd tertile) of mono-iso-butyl phthalate (tertile: ≥15.3µg/l, β=-18.3, 95% CI: -35.4, -1.2) and monobenzyl phthalate (tertile: ≥30.3µg/l, β=-17.3, 95% CI: -34.1, -0.4) had lower blood glucose levels at the time of GDM screening after adjustment for urinary creatinine and demographic covariates. | |

**BACKGROUND INFORMATION FOR TABLE 35C: OTHER STUDIES RELEVANT FOR TOXICITY ON SEXUAL FUNCTION AND FERTILITY**

| Summary of Study | Reference |
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| **Type/Objective:** Investigations of structure-activity relationships of phthalates in the pubertal-rat model<br>**Conclusion:** Testicular toxicity in the rat induced by phthalates is related to the side chain on the phthalate. The ester side-chain length of linear-chain phthalates needed to be four to six carbon atoms to produce testicular toxicity.<br>**Method:**<br>**Results:** The ester side-chain length of linear-chain phthalates needed to be four to six carbon atoms to produce testicular toxicity. Di-*n*-pentyl phthalate was the most potent in producing testicular toxicity. Phthalates of one to three carbons (methyl, ethyl, and *n*-propyl) did not produce testicular toxicity when given at a dose equimolar with DBP at 2 g/kg-d. Similarly, linear chain phthalates of seven or eight carbons did not produce adverse effects. DEHP, which has eight carbons and a branched structure, had activity more similar to that of di-*n*-hexyl phthalate than to its linear isomer di-*n*-octyl phthalate. | Foster et al, 1980 |
| **Type/Objective:** 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) are involved in the reactions that culminate in androgen biosynthesis in Leydig cells. In this study, inhibitory activities on these enzymes of 14 different phthalates with various carbon numbers in the ethanol moiety were tested.<br>**Conclusion:** Results showed that there are clear structure-activity responses for phthalates in the inhibition of both 3β-HSD and 17β-HSD3 activities (particularly the length of carbon chains in the ethanol moieties of phthalates). DBP had one the lowest half maximal inhibitory concentrations.<br>**Method:** Human and rat testis microsomes were used to investigate the inhibitory potencies on 3β-HSD and 17β-HSD3 activities of 14 different phthalates with various carbon numbers in the ethanol moiety.<br>**Results:** The results demonstrated that the half-maximal inhibitory concentrations (IC(50)s) of dipropyl (DPrP), dibutyl (DBP), dipentyl (DPP), bis(2-butoxyethyl) (BBOP) and dicyclohexyl (DCHP) phthalate were 123.0, 24.1, 25.5, 50.3 and 25.5μM for human 3β-HSD activity, and 62.7, 30.3, 33.8, 82.6 and 24.7μM for rat 3β-HSD activity, respectively. However, only BBOP and DCHP potently inhibited human (IC(50)s, 23.3 and 8.2μM) and rat (IC(50)s, 30.24 and 9.1μM) 17β-HSD3 activity. Phthalates with 1-2 or 7-8 carbon atoms in ethanol moieties had no effects on both enzyme activities even at concentrations up to 1mM. The mode of action of DCHP on 3β-HSD activity was competitive with the substrate pregnenolone but noncompetitive with the cofactor NAD+. The mode of action of DCHP on 17β-HSD3 activity was competitive with the substrate androstenedione but noncompetitive with the cofactor NADPH. | Yuan et al, 2012 |
| **Type/Objective:** To evaluate the effects of DBP/MBP on steroidogenesis in the murine Leydig tumor cell line MLTC-1 in vitro. | Chen X et al, 2013 |

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| **Conclusion:** Alterations of the steroidogenic enzymes and INSL3 in MLTC-1 cells may be involved in the biphasic effects of DBP/MBP on androgen production.<br>**Method:** MLTC-1 cells were incubated with various concentrations of DBP (100, 1, 0.01, and 0μmol/l in DMSO) and MBP (1000, 10, 0.1, and 0μmol/l in DMSO) for 24h.<br>**Results:** Testosterone secretion was stimulated at the lowest doses and inhibited at higher treatment doses of DBP and MBP. The mRNA levels of the side-chain cleavage enzyme (P450scc), cytochrome p450c17 (P450c17) and 3β-hydroxy-steroid dehydrogenase (3βHSD) were significantly reduced in the phthalate-exposed groups, whereas, the transcription and translation of insulin-like hormone 3 (INSL3) was affected by DBP and MBP. | |
| **Type/Objective:** To show that Drosophila melanogaster, an invertebrate recapitulates male reproductive toxicity phenotypes observed in mammals with DBP.<br>**Conclusion:** Effects of DBP on the male reproductive system in the fruit fly were comparable to those in mammals.<br>**Method:**<br>**Results:** Analogous to mammals, exposure to DBP reduced fertility, sperm counts, seminal proteins, increased oxidative modification/damage in reproductive tract proteins and altered the activity of a hormone receptor (estrogen related receptor) in Drosophila males. In addition, we show here that DBP is metabolized to MBP in exposed Drosophila males and that MBP is more toxic than DBP, as observed in higher organisms. | Misra et al, 2014 |

**BACKGROUND INFORMATION FOR TABLE 36A: ANIMAL STUDIES ON ADVERSE EFFECTS ON DEVELOPMENT OF THE OFFSPRING**

| Summary of Study | Reference |
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| **Type/Objective:** Developmental toxicity study with DBP in mice<br>**Conclusion:** The NOAEL for maternal, teratogenic and embryotoxic effects was 0.05% in the diet (~100 mg/kg bw).<br>**Method:** Lowest dose level administered to mice (ICR-JCL strain) was 0.005% in the diet during day 1-18 of gestation. Next higher dose levels were 0.05 and 0.5% in the diet (equal to 100 and 400 mg/kg bw).<br>**Results:** Number of spontaneous abortions and number of mice with live offspring were not different from controls in any treated group. At 0.5% in the diet maternal toxicity (increased kidney wts) and embryotoxicity (lower no. of live offspring) were observed. In addition teratogenic effects were induced at 0.5% as was demonstrated by a statistically significantly higher incidence of external anomalies (non-closing eye-lid, encephalocele, cleft palate, spina bifida). Also a higher (but not statistically significantly) incidence of skeletal anomalies, especially of sternum, was seen at this dose-level. The rate of ossification was normal in all treated groups. | Hamano et al, 1977, as summarized in EC, 2003 |
| **Type/Objective:** Dietary developmental toxicity study in mice<br>**Conclusion:** The dose-level of 0.2% in the diet (~350 mg/kg bw) is a NOAEL for embryotoxicity. NOAEL for maternal toxicity and teratogenicity was 0.4% in the diet (ca. 660 mg/kg bw).<br>**Method:** Mice (ICL-ICR strain) received 0.05, 0.1, 0.2, 0.4 or 1.0% DBP in their diet (ca. 80, 180, 350, 660 and 2,100 mg/kg bw) during days 1-18 of pregnancy.<br>**Results:** Maternal growth was statistically significantly reduced at 1.0%. Fetal mortality and no. of resorptions were increased at dose-levels from 0.1% onwards, but statistically significant at 1.0% only and without any dose-relationship. No. of corpora lutea and implantations were normal. Fetal wts were decreased in all treated groups, but statistically significant at 1.0 and 0.4% only. In all treated groups the incidence of skeletal variations was higher (lumbar ribs) and ossification was statistically significantly retarded as shown by the lower number of ossified coccygia. The effect on the fetal weights at the lower three dose-levels and the effect on the incidences of skeletal variations at all dose-levels can be attributed to the relatively low litter size in the control group. Limited evidence for teratogenicity was seen in this study at 1.0%. At this dose-level only 2 male and 1 female fetus survived and 2 out of these 3 survivors showed exencephaly. | Shiota et al, 1980, as summarized in EC, 2003<br><br>Comment – Original abstract was not found and effect levels in the summary in EC (2003) are inconsistent. |
| **Type/Objective:** Dietary developmental toxicity study in mice<br>**Conclusion:** A high dose of DBP (~2100 mg/kg bw) might be embryotoxic and teratogenic.<br>**Method:** DEHPand DBP were mixed with diet at graded levels of 0.05, 0.1, 0.2. 0.4 and 1.0 wt-% and given to pregnant ICR mice throughout gestation.<br>**Results:** Maternal weight gain was suppressed and fetal resorption increased at 1.0% DBP (~2100 mg/kg bw). External malformations at 1.0% DBP showed | Shiota and Nishimura, 1982 |

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| borderline significance. The major malformations in treated groups were neural tube defects (exencephaly and myeloschisis), suggesting that the phthalic acid esters (PAEs) affect neural tube closure in developing embryos. Treatment with the compounds caused intrauterine growth retardation and delayed ossification with an apparently dose-related response pattern. | |
| **Type/Objective:** Developmental toxicity study in Wistar rats<br>**Conclusion:** 500 mg/kg bw is a LOAEL in this study for maternal toxicity and embryotoxicity. For teratogenic effects 500 mg/kg bw is a NOAEL<br>**Method:** 500, 630, 750 or 1,000 mg DBP/kg bw was given by gavage during day 7-15 of pregnancy.<br>**Results:** A dose-related increased incidence of animals with reddish-brown staining of facial fur and piloerection was seen. Maternal death (2/11) occurred at 1,000 mg/kg bw. Maternal body weight gain was decreased at all dose-levels with a dose relationship, statistically significant at doses of 630 mg/kg bw and higher. Food consumption showed a statistically significant decrease during gestation at 750 and 1,000 mg/kg bw. No. of implantations/litter was normal. Complete resorption of implanted embryos was seen in all animals at 1,000 mg/kg bw and in 10/12 at 750 mg/kg bw. At 630 and 500 mg/kg bw 2/12 and 2/11 litters, respectively, were completely resorbed. In control group none of the litters was resorbed. Statistically significantly higher numbers of resorptions and dead fetuses/litter, higher incidences of postimplantation loss/litter and statistically significantly lower numbers of live fetuses/litter were noted at doses of 630 mg/kg bw and above. At 500 mg/kg bw, no. of resorptions and dead fetuses/litter and postimplantation loss were still increased but not statistically significant. Also the number of live fetuses/litter was still lower at 500 mg/kg bw but not statistically significant. Statistically significantly lower fetal wts were seen at 750 and 630 mg/kg bw and also at 500 mg/kg bw the fetal wt. was lower but not statistically significant. The incidences of fetuses with external malformations were higher at 630 and 750 mg/kg bw, statistically significant at 750 mg/kg bw. Cleft palate was predominantly observed. The number of fetuses with skeletal malformations was higher at 630 mg/kg bw, but not statistically significant (predominantly fused sternebrae and cervical vertebral arches). At 750 mg/kg bw too few fetuses were available for skeletal examination. | Ema et al, 1993, as summarized in EC, 2003 |
| **Type/Objective:** Developmental toxicity study in Wistar rats<br>**Conclusion:** Susceptibility to the teratogenicity of DBP varied with the developmental stage at dosing. With gavage doses of 750, 1000, or 1500 mg DBP/kg on GD 7-9, 10-12, or 13-15, the highest incidence of malformed fetuses occurred after treatment with DBP on days 13-15.<br>**Method:** 750, 1000, or 1500 mg DBP/kg was given by gastric intubation to pregnant female rats on GD 7-9, 10-12, or 13-15.<br>**Results:** Postimplantation loss was 100% for each period of dosing at 1500 mg/kg. Postimplantation loss was significantly increased at 750 and 1000 mg/kg regardless of the days of treatment. No evidence of teratogenicity was detected when DBP was given on GD 10-12. Treatment on GD 7-9 with 750 or 1000 mg/kg caused a significant increase in the number of skeletal malformations (e.g. deformity of the vertebral column in the cervical and thoracic regions and of the ribs. Doses of 750 or 1000 mg/kg on GD 13-15 resulted in significantly increased incidence of fetuses with external and skeletal malformations such as cleft palate and fusion of the sternebrae. The frequency of malformations increased with dose. | Ema et al, 1994 |

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| **Type/Objective:** Determination of maximum perinatal exposure (MPE) in F344/N rats<br>**Conclusion:** Although epididymal hypospermia started at a dose of 5,000 ppm (0.5%), it was not life threatening and 10,000 ppm (1.0%) was recommended as the MPE concentration for male and female rats.<br>**Method:** DBP was administered in the diet to dams during gestation and lactation and to the pups postweaning for four additional weeks. Concentrations were 0, 1,250, 2,500, 5,000, 7,500, 10,000, and 20,000 ppm.<br>**Results:** Decreased weight gains were noted in dams exposed to 20,000 ppm during gestation and to dams exposed to 10,000 ppm during lactation. The gestation index (number of live pups per breeding female) was significantly lower in the 20,000 ppm group than in the controls, and pup mortality in this group was marked (100% by Day 1 of lactation); however, survival was 89% or greater in all other treatment groups. The mean body weight of pups in the 10,000 ppm group at Day 28 of lactation was approximately 90% of the mean weight of control pups. Pups were weaned onto diets containing dibutyl phthalate at the same concentrations fed to dams. After an additional 4 weeks of dietary administration, final mean body weights of pups in the 10,000 ppm groups were 92% of the control value for males and 95% of the control value for females. Hepatomegaly (increased relative liver weight) was observed in males in all exposed groups and in females receiving 2,500 ppm or greater. No gross lesions were observed at necropsy. Moderate hypospermia of the epididymis was diagnosed in all male rats in the 7,500 and 10,000 ppm groups; mild hypospermia of the epididymis was diagnosed in 2 of 10 males in the 5,000 ppm group. No degeneration of the germinal epithelium was detected in the testis of these rats. | NTP, 1995 |
| **Type/Objective:** Determination of maximum perinatal exposure (MPE) in C3H male mice and C57BL/6 female<br>**Conclusion:** Developmental toxicity and fetal and pup mortality were suggested at $\geq$ 7,500 ppm. Thus a MPE concentration of 5,000 ppm was suggested by the data.<br>**Method:** Dams received 0, 1,250, 2,500, 5,000, 7,500, 10,000, or 20,000 ppm DBP in feed during gestation and lactation; pups were weaned onto the same diets as the dams received and were exposed for an additional 4 weeks.<br>**Results:** The gestation period was longer in dams that received 2,500 ppm or greater than in the controls, and gestational body weight gain depressions were noted in dams receiving 7,500 ppm or greater. Only 5 of 20 females in the 10,000 ppm group delivered live pups, and none of the 20 females receiving 20,000 ppm delivered live pups. Only one pup in the 10,000 ppm group survived past Lactation Day 1; the number of live pups per litter in the 7,500 ppm group also remained low throughout lactation. No deaths of either male or female pups occurred after weaning. Initial (postweaning) and final body weights of male pups receiving 2,500 ppm or greater were significantly less than those of the control group. The mean body weights of exposed female pups were similar to the control body weight at weaning and remained similar throughout the 4 weeks postweaning. Hepatomegaly was present in male mice in all exposed groups, and the absolute liver weight of males administered 7,500 ppm was greater than that of the controls; although a similar change was apparent in females, no statistical differences between the liver weights of exposed and control females were detected. No treatment-related gross lesions were identified at necropsy, and no histopathologic lesions definitively associated with treatment were observed in male or female mice in the 7,500 ppm groups. The one surviving male pup in the | NTP, 1995 |

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| 10,000 ppm group had cytoplasmic alteration in the liver, consistent with peroxisome proliferation. | |
| **Type/Objective:** Developmental toxicity study in CD rats<br>**Conclusion:** Exposure to high doses of DBP in utero and during the entire lactational period induced profound reproductive tract malformations in rats.<br>**Method:** Pregnant dams were dosed by gavage at 0, 250, 500 or 750 mg DBP/kg BW /day from GD 3 throughout pregnancy and lactation until the offspring were at postnatal day (PND) 20.<br>**Results:** Maternal body weights throughout the dosing period were not significantly affected by DBP treatment, and no clinical signs of toxicity were observed. No effect on parturition was apparent. Litter size was decreased at 750 mg/kg/day. The number of implantation sites (on PND 21), proportion of pups born alive, sex ratio of live pups, and weight at birth were comparable in all groups. Adverse effects on the male reproductive system were induced in a dose-dependent manner. Anogenital distance on PND 2 was significantly decreased in males from dams treated with 500 and 750 mg DBP/kg/day. Undescended testes on PND 40 were observed in 0, 13, 29, and 25% of litters at 0, 250, 500, and 750 mg/kg/day, respectively. Small malformed prepuces and penises occured in 86 and 50% of litters at 500 and 750 mg/kg/day, respectively. Decreased testicular size and poorly developed or absent epididymis were observed in all DBP groups. Lack of patent vagina and malformed or absent uteri and ovaries occured at 500 and 750 mg DBP/kg/day. | Mylchreest and Foster, 1997 |
| **Type/Objective:** One high dose of DBP was given to pregnant rats to determine the time of susceptibility to teratogenicity.<br>**Conclusion:** DBP induced two discrete responses on GD 8 (cervical vertebrae), GD 9 (cervical and thoracic vertebrae, ribs, and renal pelvis), and GD 15 (cleft palate and fusion of sternebrae).<br>**Method:** One dose of 1500 mg DBP/kg was given on one of GD 6-16. Fetuses were evaluated on GD 20.<br>**Results:** Significant increases in incidences of fetuses with skeletal malformations, of fetuses with skeletal and internal malformations and of fetuses with external and skeletal malformations were noted after dosing on GD 8, 9, and 15, respectively. Deformity of the cervical vertebrae was frequently observed after administration on GD 8. Deformity of the cervical and thoracic vertebrae and ribs and dilatation of the renal pelvis were predominantly found in fetuses of dams treated on GD 9. Cleft palate and fusion of the sternebrae were exclusively detected after administration on GD 15. | Ema et al, 1998a |
| **Type/Objective:** Dietary developmental toxicity study with DBP in Wistar rats<br>**Conclusion:** The NOAEL in this study is 0.5% DBP in the diet (~331 mg/kg bw).<br>**Method:** Pregnant rats received a diet with 0, 0.5, 1.0 or 2.0% DBP (~0, 331, 555 or 661 mg/kg bw, respectively) during day 11- 21 of gestation. The dams were killed on day 21 of pregnancy.<br>**Results:** Body weight gain and food consumption of dams during treatment period was decreased significantly at 1.0 and 2.0% DBP in the diet with a dose relationship. No postimplantation loss, no changes in number of live fetuses, number of resorptions or number of dead fetuses were seen. At 2.0% weights of male and female fetuses were significantly decreased. An increased incidence of fetuses with cleft palate and fusion of the sternebrae were seen at 2.0% in the diet. At 1.0 and 2.0% in the diet the number of male fetuses with undescended testes (internal malformation) and decreased anogenital distance was increased. Anogenital distance of female fetuses in the treated groups was comparable to | Ema et al, 1998b, as summarized in EC, 2003 |

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| control values. | |
| **Type/Objective:** Developmental toxicity study in CD rats with DBP given by gavage with the goal of comparing the activity of DBP to that of the antiandrogen flutamide (FLU).<br>**Conclusion:** The androgen signaling necessary for male sexual differentiation was disrupted by DBP and FLU. However, the many phenotypic differences between DBP and FLU, particularly the lack of sensitivity of the developing prostate to DBP, indicate that DBP is not a classical AR antagonist like FLU.<br>**Method:** It was known that gestational and lactational exposure of rats to DBP at ≥250 mg/kg/day causes reproductive tract malformations and testicular toxicity in the adult male offspring. Although this disruption of androgen-regulated sexual differentiation indicates an antiandrogenic mechanism, DBP and its biologically active monoester metabolite do not bind to the androgen receptor (AR) in vitro. Pregnant rats received by gavage either the FLU at 100 mg/kg/day (n = 5) or DBP at 0, 100, 250, or 500 mg/kg/day (n = 10) on GD 12-21. Results with FLU were then compared to those with DBP.<br>**Results:** In this study, all males at sexual maturity, all males in the FLU group had hypospadias with a vaginal pouch, no prostate, no epididymis or vas deferens. In contrast to FLU, the prostate was absent in only 6% of males at 500 mg DBP/kg/day, and males with hypospadias had no vaginal pouch (40% of males at 500 mg/kg/day). The epididymis was absent in 10 and 50% of males at 250 and 500 mg DBP/kg/day, respectively, and no vas deferens was found at these dose levels in 2 and 27% of DBP-exposed males. DBP produced abdominal testes at a low incidence (2 and 10% of males at 250 and 500 mg/kg/day, respectively), whereas FLU produced inguinal testes in 64% of males. No malformations were observed at 100 mg DBP/kg/day, but preputial separation was delayed at all DBP dose levels. In the testis, FLU and DBP (250 and 500 mg DBP/kg/day) caused degeneration of the seminiferous epithelium, whereas DBP caused interstitial cell hyperplasia, adenoma (two males), and increased AR immunostaining at 500 mg/kg/day. In conclusion, prenatal male sexual differentiation is the sensitive period for the reproductive and developmental toxicity of DBP. | Mylchreest et al, 1998a |
| **Type/Objective:** Developmental toxicity study in rats with emphasis on effects on male pups<br>**Conclusion:** In the male offspring, DBP produced the same spectrum of effects elicited by the antiandrogen flutamide. That is, DBP specifically impaired the androgen-dependent development of the male reproductive tract, suggesting that DBP is not estrogenic but antiandrogenic in the rat at these high dose levels.<br>**Method:** Pregnant CD rats (n = 10) were given DBP at 0, 250, 500, or 750 mg/kg/day (p.o.) throughout pregnancy and lactation until their offspring were at postnatal day 20. Maternal body weights throughout the dosing period were comparable in all groups.<br>**Results:** At 750 mg/kg/day, the number of live pups per litter at birth was decreased and maternal effects on pregnancy and postimplantation loss are likely to have occurred. Anogenital distance was decreased at birth in the male offspring at 500 and 750 mg/kg/day. The epididymis was absent or underdeveloped in 9, 50, and 71% of adult offspring (100 days old) at 250, 500, and 750 mg/kg/day, respectively, and was associated with testicular atrophy and widespread germ cell loss. Hypospadias occurred in 3, 21, and 43% of males and ectopic or absent testes in 3, 6, and 29% of males at 250, 500, and 750 mg/kg/day, respectively. Absence of prostate gland and seminal vesicles as well as small testes and seminal vesicles were noted at 500 and 750 mg/kg/day. Vaginal opening and estrous | Mylchreest et al, 1998b |

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| cyclicity, both estrogen-dependent events, were not affected in the female offspring, although low incidences of reproductive tract malformations were observed at 500 and 750 mg/kg/day. | |
| **Type/Objective:** Developmental toxicity study to determine a NOAEL for effects of DBP in the CD rat<br>**Conclusion:** The NOAEL was 50 mg/kg/day for this 10-day prenatal exposure to DBP, currently the lowest NOAEL for the toxicity of DBP. The early changes in the fetal testis suggest that this organ is the primary target of the reproductive and developmental toxicity of DBP via disruption of androgen-dependent differentiation processes.<br>**Method:** Adult rats exposed from GD 12 to 21 to DBP have decreased sperm production, interstitial cell hyperplasia and adenomas, as well as reproductive tract malformations. Previous studies using high doses failed to establish a NOAEL. The pattern of effects resembles that elicited by antiandrogens, but DBP does not interact directly with the androgen receptor (AR). An indirect mechanism is proposed through which DBP alters androgen-dependent male sexual differentiation by disrupting the androgen status in the fetal testis. The aims of the present study were to determine a NOAEL for the male reproductive and developmental toxicity of DBP and to characterize changes in morphology and androgen status in the fetal reproductive tract. Pregnant CD rats were given DBP by gavage at 0, 0.5, 5, 50, 100 (n = 19-22), or 500 mg/kg/day (n = 11) from GD 12 to 21.<br>**Results:** Absent epididymis and vas deferens, hypospadias, interstitial cell hyperplasia and adenoma, small reproductive organs, and decreased anogenital distance occurred only at the highest dose. Retained areolas or nipples in neonates and seminiferous tubule degeneration in the adult testis were present at 100 and 500 mg/kg/day but not at lower doses. The fetal testis and epididymis were examined on GD 21 following exposure to 500 mg DBP/kg/day. In the testicular interstitial compartment, DBP increased cell numbers and AR immunostaining. Seminiferous tubules also contained multinucleated gonocytes. DBP reduced the number of epididymal ducts and the intensity of AR staining in some of these ducts. Androgen levels in the fetal testis were decreased by DBP. | Mylchreest et al, 1999a |
| **Type/Objective:** A developmental toxicity study was performed in rats to compare the effects of DBP and the antiandrogen flutamide using a shorter exposure during the prenatal period of male sexual differentiation in rats. The study was performed because, although the disruption of male rat reproductive development and function by DBP given during gestation and lactation indicates an antiandrogenic mechanism, DBP and its biologically active metabolite do not interact with the androgen receptor (AR) in vitro.<br>**Conclusion:** Thus prenatal male sexual differentiation is a sensitive period for the reproductive toxicity of DBP. A NOAEL was not established and the LOAEL was 100 mg/kg/day. Flutamide and DBP disrupted the androgen signaling necessary for male sexual differentiation but with a different pattern of antiandrogenic effects.<br>**Method:** Pregnant CD rats received DBP at 0, 100, 250, or 500 mg/kg/day po (n = 10) or flutamide at 100 mg/kg/day po (n = 5) from Gestation Days 12 to 21.<br>**Results:** In F1 males, DBP (500 mg/kg/day) and flutamide caused hypospadias; cryptorchidism; agenesis of the prostate, epididymis, and vas deferens; degeneration of the seminiferous epithelium; and interstitial cell hyperplasia of the testis. Flutamide and DBP (250 and 500 mg/kg/day) also produced retained thoracic nipples and decreased anogenital distance. Interstitial cell adenoma | Mylchreest et al, 1999b |

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| occurred at 500 mg DBP/kg/day in two males. The only effect seen at 100 mg DBP/kg/day was delayed preputial separation. In contrast to flutamide, DBP caused a low incidence of prostate agenesis and hypospadias with no vaginal pouch. The low incidence of DBP-induced intraabdominal testes contrasted with the high incidence of inguinal testes seen with flutamide. | |
| **Type/Objective:** A dietary developmental toxicity study was performed in CD rats with 0.5, 1.0, or 2.0% DBP in the diet on GD 11-21.<br>**Conclusion:** DBP given during the second half of pregnancy produces adverse effects on reproductive development in male fetuses.<br>**Method:** Pregnant rats were fed a diet containing DBP at 0 (control), 0.5, 1.0 or 2.0% ad libitum on GD 11-21. Pregnant rats were sacrificed on GD 21, and their fetuses were examined.<br>**Results:** Body weight gain and food consumption of dams were decreased at 1.0 and 2.0% DBP. Numbers of live fetuses, resorptions, and dead fetuses were not affected by treatment. The weights of fetuses at 2.0% were significantly decreased in both sexes. The incidences of fetuses with cleft palate and fetuses with fusion of the sternebrae at 2.0% and fetuses with undescended testes at 1.0 and 2.0% were significantly increased. There was a significant decrease in the anogenital distance (AGD) of male fetuses in the 1.0 and 2.0% groups. AGD of female fetuses in the DBP-treated groups was comparable to that in the control group. | Ema et al, 1999 |
| **Type/Objective:** 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.<br>**Conclusion:** 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.<br>**Method:** 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.<br>**Results:** 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% ($p < 0.01$), respectively, versus 0% in other groups). All three of the phthalate treatments that induced areolas also | Gray et al, 2000 |

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| induced a significant incidence of reproductive malformations. The percentages of males with malformations were 82% ($p < 0.0001$) for **DEHP**, 84% ($p < 0.0001$) for BBP, and 7.7% ($p < 0.04$) in the DINP group. | |
| **Type/Objective:** A developmental toxicity study was performed to establish as NOAEL for alterations in male reproductive development and function in CD rats with maternal exposure on GD 12-21.<br>**Conclusion:** The NOAEL and LOAEL were 50 and 100 mg/kg/day, respectively.<br>**Method:** Pregnant CD rats were given DBP by gavage at 0, 0.5, 5, 50, or 100 mg/kg/day (n = 19-20) or 500 mg/kg/day (n = 11) from gestation day 12 to 21.<br>**Results:** In male offspring, anogenital distance was decreased at 500 mg DBP/kg/day. Retained areolas or nipples were present in 31 and 90% of male pups at 100 and 500 mg/kg/day, respectively. Preputial separation was not delayed by DBP treatment in males with normal external genitalia, but cleft penis (hypospadias) was observed in 5/58 rats (4/11 litters) at 500 mg/kg/day. Absent or partially developed epididymis (23/58 rats in 9/11 litters), vas deferens (16/58 animals in 9/11 litters), seminal vesicles (4/58 rats in 4/11 litters), and ventral prostate (1/58 animals) occurred at 500 mg/kg/day. In 110-day-old F(1) males, the weights of the testis, epididymis, dorsolateral and ventral prostates, seminal vesicles, and levator ani-bulbocavernosus muscle were decreased at 500 mg/kg/day. At 500 mg/kg/day, widespread seminiferous tubule degeneration was seen in 25/58 rats (in 9/11 litters), focal interstitial cell hyperplasia in 14/58 rats (in 5/11 litters), and interstitial cell adenoma in 1/58 rats (in 1/11 litters). | Mylchreest et al, 2000 |
| **Type/Objective:** A developmental toxicity study was performed in rats.<br>**Conclusion:** Based in part on parallel work with DBP-dosed pseudopregnant rats, the authors concluded that early embryonic loss due to DBP may be mediated, at least in part, via the suppression of uterine decidualization, an impairment of uterine function.<br>**Method:** 250, 500, 750, 1,000, 1,250 or 1,500 mg DBP/kg/day (p.o.) on days 0-8 of pregnancy (sperm = day 0 of pregnancy) and pregnancy outcome was determined on day 20 of pregnancy.<br>**Results:** DBP caused significant increases in incidences of preimplantation loss at 1,250 mg/kg/day and above and of postimplantation loss in females having implantations at 750 mg/kg/day and above. A significantly lower weight of the uterus, which indicates suppression of uterine decidualization, was found in given DBP at 750 mg/kg/day and above. | Ema et al, 2000a |
| **Type/Objective:** A developmental toxicity study was performed in rats to determine the susceptible days for the adverse effects of DBP on development of reproductive system in male offspring following maternal administration on successive 3-day period during late pregnancy.<br>**Conclusion:** The period of days 15-17 of pregnancy was the most susceptible for DBP-induced undescended testes and decreased AGD in male offspring.<br>**Method:** Pregnant rats were given DBP by gastric intubation at 1000 or 1500 mg/kg on days 12-14 or 18-20 of pregnancy or at 500, 1000 or 1500 mg/kg on days 15-17 of pregnancy.<br>**Results:** A significant decrease in the maternal body weight gain and/or food consumption was found in the DBP-treated groups regardless of the days on which DBP at 1000 and 1500 mg/kg was given. A significant increase in the number of resorptions per litter was found in the groups given DBP at 1500 mg/kg on days 12-14 and 15-17 of pregnancy. The weights of male and female fetuses were significantly decreased in the groups given DBP at 1000 and 1500 mg/kg on days 12-14 and 18-20 and at 1500 mg/kg on days 15-17. A significant increase in | Ema et al, 2000b |

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| the incidence of fetuses with undescended testes was found at 1500 mg/kg on days 12-14 and at all doses on days 15-17. A significant decrease in the anogenital distance (AGD) of male fetuses was observed in the groups treated with DBP regardless of the days of treatment. The AGD/body weight ratio in male fetuses was significantly reduced in the groups given DBP on days 15-17, but neither on days 12-14 nor 18-20. The AGD of female fetuses in the DBP-treated groups was comparable to that in the control group. | |
| **Type/Objective:** The effects of DBP on reproductive function were investigated in a developmental toxicity study using both pregnant and pseudopregnant rats.<br>**Conclusion:** These findings suggest that early embryonic loss due to DBP may be mediated, at least in part, via the suppression of uterine decidualization, an impairment of uterine function.<br>**Method:** Rats were given DBP by gastric intubation at 0, 250, 500, 750, 1000, 1250 or 1500 mg/kg on Days 0 to 8 of pregnancy and the pregnancy outcome was determined on Day 20 of pregnancy. The same doses of DBP were given to pseudopregnant rats, with an induced decidual cell response, on Days 0 to 8 of pseudopregnancy, and the uterine weight on Day 9 served as an index of the uterine decidualization.<br>**Results:** DBP caused significant increases in the incidences of preimplantation loss in females successfully mated at 1250 and 1500 mg/kg and of postimplantation loss in females having implantations at 750 mg/kg and above. The uterine decidualization in pseudopregnant rats was significantly decreased at 750 mg/kg and above. | Ema et al, 2000c |
| **Type/Objective:** A developmental toxicity study was performed to determine the susceptible days for the adverse effects of DBP on the development of reproductive system in male offspring during late pregnancy.<br>**Conclusion:** We suggest that the period of days 15-17 of pregnancy was the most susceptible for DBP-induced decreased AGD and undescended testes in male offspring.<br>**Method:** Pregnant rats were given DBP by gastric intubation at 1000 or 1500 mg/kg on days 12-14 or days 18-20 of pregnancy or at 500, 1000 or 1500 mg/kg on days 15-17 of pregnancy. Pregnancy outcome was determined on day 21 of pregnancy.<br>**Results:** The maternal body weight gain and/or food consumption was significantly decreased in the DBP-treated groups regardless of the days on which DBP at 1000 and 1500 mg/kg was given. A significantly higher incidence of postimplantation loss was observed in rats given DBP at 1500 mg/kg on days 12-14 and days 15-17 of pregnancy. The body weights of male and female fetuses were significantly decreased after the administration of DBP at 1000 and 1500 mg/kg on days 12-14 and days 18-20 and at 1500 mg/kg on days 15-17. A significant decrease in the AGD of male fetuses was found after the administration of DBP regardless of the days of administration. The AGD of female fetuses in the DBP-treated groups was comparable to that in the control group. The AGD/body weight ratio in male fetuses was significantly reduced in the groups given DBP on days 15-17, but there was no reduction in the groups given DBP on days 12-14 or days 18-20. The incidence of fetuses with undescended testes was significantly increased at 1500 mg/kg on days 12-14 and at all doses on days 15-17. | Ema and Miyawaki, 2001 |
| **Type/Objective:** DBP acts as an antiandrogen by decreasing fetal testicular testosterone synthesis when male rats are exposed in utero. DBP-exposed male rats develop malformations of the reproductive tract secondary to the reduced | Barlow and Foster, 2003 |

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| fetal androgen levels. However, these malformations and the associated histologic lesions have only been described in adult rats. The objective of this study was to describe the male reproductive tract lesions in fetal, early postnatal, and young adult male rats following DBP exposure in utero.<br>**Conclusion:** As the animals were only dosed in utero, these findings indicate that DBP can initiate fetal testicular and epididymal changes that may not manifest as clear malformations until adulthood.<br>**Method:** Pregnant Sprague-Dawley rats were exposed to 500 mg/kg/day DBP by gavage on gestation days (GD) 12 to 21. Male reproductive tracts were examined on GD 16 to 21 and on postnatal days (PND) 3, 7, 16, 21, 45, and 70.<br>**Results:** In the fetal testes, large aggregates of Leydig cells, multinucleated gonocytes, and increased numbers of gonocytes were first detected on GD 17 and increased in incidence to 100% by GD 20 and 21. These lesions resolved during the early postnatal period, while decreased numbers of spermatocytes were noted on PND 16 and 21. On PND 45, there was mild degeneration of the seminiferous epithelium, which progressed to severe seminiferous epithelial degeneration on PND 70. On PND 70, the degeneration was concurrent with ipsilateral malformed epididymides, which caused obstruction of testicular fluid flow and secondary pressure atrophy in the seminiferous tubules. In the fetus, the epididymal lesion was observed as decreased coiling of the epididymal duct. The decreased coiling progressed into the early postnatal period and adulthood, at which time malformed epididymides were apparent. | |
| **Type/Objective:** In a dietary developmental toxicity with rats, dams received 20 to 10,000 ppm DBP from GD 15 to PND 21.<br>**Conclusion:** Developmental exposure to DBP affected female sexual development involving pituitary function, while in males testicular toxicity was mostly reversible but mammary gland toxicity (degeneration and atrophy of mammary gland alveoli) was persistent at a dose level as low as 20 ppm (1.5-3.0 mg/kg/d).<br>**Method:** Maternal rats were given DBP at dietary concentrations of 0, 20, 200, 2000 and 10,000 ppm from gestational day 15 to postnatal day (PND) 21.<br>**Results:** At 10,000 ppm, male offspring showed a decreased neonatal AGD and retention of nipples (PND 14). At PND 21, reduction of testicular spermatocyte development was evident from 20 ppm, as well as mammary gland changes at low incidence in both sexes. At this time point, population changes of pituitary hormone-immunoreactive cells were observed at 10,000 ppm with a similar pattern of increase in the percentages of luteinizing hormone (LH)-positive and decrease in follicle-stimulating hormone (FSH) and prolactin producing cells in both sexes, effects also being evident on FSH from 200 ppm and LH from 2000 ppm in females. During postnatal week (PNW) 8-11, marginal increase of the number of cases with extended diestrus was found at 10,000 ppm. At adult stage necropsy, testicular lesions appeared to be very faint in most cases, but degeneration and atrophy of mammary gland alveoli were observed in males from 20 ppm. The proportion of FSH-positive cells in the pituitaries at PNW 11 was increased in both sexes at 10,000 ppm. | Lee et al, 2004 |
| **Type/Objective:** In utero exposure of male rats to the antiandrogen DBP) leads to decreased anogenital distance (AGD) on postnatal day (PND) 1, increased areolae retention on PND 13, malformations in the male reproductive tract, and histologic testicular lesions including marked seminiferous epithelial degeneration and a low incidence of Leydig cell (LC) adenomas on PND 90. One objective of this developmental toxicity study in rats was to determine the incidence and | Barlow et al, 2004 |

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| persistence of decreased AGD, increased areolae retention, and LC adenomas in adult rats following in utero DBP exposure. A second objective was to determine whether AGD and areolae retention during the early postnatal period are associated with lesions in the male reproductive tract.<br>**Conclusion:**<br>**Method:** Pregnant Crl:CD(SD)BR rats were gavaged with corn oil or DBP at 100 or 500 mg/kg/day, 10 dams per group. Three replicates of rats (n = 30 rats per replicate) were exposed from gestation day 12 to 21 and the male offspring allowed to mature to 6, 12, or 18 months of age.<br>**Results:** Gross malformations in the male reproductive tract and histologic lesions in the testes were similar to those previously described. However, testicular dysgenesis, a lesion of proliferating LCs and aberrant tubules that has not been previously described in DBP-exposed testes, was diagnosed. The incidence of this lesion was approximately 20% unilateral and 7-18% bilateral in the high-dose group and was similar among all ages examined, implicating a developmental alteration rather than an age-related change. AGD and areolae retention were found to be permanent changes following in utero exposure to 500 mg/kg/day of DBP. Decreased AGD was a sensitive predictor of lesions in the male reproductive tract, relatively small changes in AGD were associated with a significant incidence of male reproductive malformations. In utero DBP exposure induced proliferative developmental lesions, some of which would have been diagnosed as LC adenomas by the morphological criteria set forth by the Society of Toxicologic Pathology. However, these lesions were dissimilar to traditional LC adenomas as the LCs were poorly differentiated and the lesions contained aberrant seminiferous tubules. | |
| **Type/Objective:** A developmental toxicity study in rats<br>**Conclusion:** The NOAEL for developmental toxicity was based on pup body weight and male reproductive lesions at 50 mg/kg/day.<br>**Method:** Pregnant rats were treated with different doses of DBP (0, 50, 250, and 500 mg/kg body weight/day) by daily gavage from GD1 to PND21. The developmental condition of F1 rats and the reproductive system of mature F1 male rats were monitored.<br>**Results:** No effects on the dams were noted, but reduced reproductive parameters included birth weight, number of live pups per litter, body weight gain and male AGD. Severe damage to the reproductive system of mature F1 male rats given $\geq$250 mg/kg/day included testicular atrophy, underdeveloped or absent epididymis, undescended testes, obvious decline of epididymal sperm parameters, total sperm heads per g testis, decrease of organ/body weight ratio of epididymis and prostate. | Zhang et al, 2004 |
| **Type/Objective:** A study was conducted to evaluate male reproductive organ development in early postnatal male Sprague-Dawley rats following neonatal exposure to DBP on PND 5-14 (no *in utero* exposure).<br>**Conclusion:** These results demonstrate that neonatal exposure to DBP causes permanent changes in the endocrine system and results in abnormal male reproductive tract development up to puberty. Thus our data suggest that DBP is likely to exert its antiandrogenic actions through the disruption of AR or ERbeta expression during the early neonatal stage.<br>**Method:** Neonatal male rats were injected s.c. from days 5 to 14 after birth with corn oil (control) and DBP (5, 10, and 20 mg/animal). Animals were killed at PNDs 31 and 42 and testes, epididymis, seminal vesicles, ventral prostate, levator ani plus bulbocavernosus muscles (LABC), and cowpers glands were weighed. In | Kim et al, 2004 |

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| addition, the expressions of androgen receptor (AR), estrogen receptors (ERs), and steroidogenic factor-1 (SF-1) were also examined in the testes.<br>**Results:** Total body weights gains were significantly reduced at PND 29-31, but gradually recovered on PND 42. However, DBP (20 mg/animal) significantly reduced the weights of testes and accessory sex organs (seminal vesicles, LABC, and cowpers glands), but not of the epididymis, versus the control on PND 31. These adverse effects persisted through puberty at PND 42. DBP also slightly delayed testis descent (bilateral) in a dose-dependent manner. Serum testosterone levels did not show any significant changes in the control and DBP treatment groups. Histomorphological examination showed mild diffuse Leydig cells hyperplasia in the interstitium of severely affected tubules on PND 31. Only a few multinuclear germ cells were observed. DBP (20 mg/animal) significantly decreased the expression of AR, whereas ERbeta and SF-1 expressions were increased in a dose-dependent manner on PND 31 in the rat testes. On PND 42, DBP (20 mg/animal) significantly inhibited ERbeta expression in the testes, but not AR, ERalpha, and SF-1. | |
| **Type/Objective:** In a developmental toxicity study in Sprague-Dawley rats, dams received 500 mg/kg/day for only two successive days during the period of GD 14 to 20. Effects on male offspring were evaluated.<br>**Conclusion:** These findings suggest that two-day DBP exposure is highly detrimental to the developing reproductive tract of the male fetus and the critical window for abnormal development is GD 16-18.<br>**Method:** Pregnant dams were dosed at 500 mg/kg/day on GD 14&15, 15&16, 16&17, 17&18, 18&19, or 19&20. Anogenital distance (AGD) was measured on PND 1, and 13; while nipple number was recorded on PND 13 only. After weaning males were allowed to mature to PND 90 at which time they were necropsied. Nipple number, and AGD were recorded and testes, epididymides, seminal vesicles, prostate gland, kidneys and liver weighed. Blood serum assayed for total testosterone.<br>**Results:** No observable effects on litter size, sex ratio, or mortality of pups were noted. Serum testosterone concentrations were not biologically affected. Significant permanent reductions in AGD were seen in males exposed prenatally to DBP on GD 15&16 or GD 18&19. On PND 13 areolae were present in males exposed to DBP on GD 15&16, 16&17, 17&18, and 19&20, however significant permanent nipple retention occurred only in males after DBP exposure on GD 16&17. Exposure to DBP on only GD 17&18 elicited a significant reduction in epididymal weights; while exposure on only GD 16&17 caused a significant increase in the weights of the testes due to edema. Epididymal and testicular malformations were most prevalent after exposure to DBP on any gestational day. Epididymal malformations, characterized by agenesis of various regions, and small or flaccid testes were significantly increased in DBP exposed males only on GD 17& 18. | Carruthers and Foster, 2005a |
| **Type/Objective:** A developmental toxicity study in Sprague-Dawley rats was conducted to identify the critical days for the abnormal development of the male reproductive tract, specifically the testis and epididymis.<br>**Conclusion:** These findings suggest that 2-day DBP exposure is highly detrimental to the developing reproductive tract of the male fetus and the critical window for abnormal development is GD 16-18.<br>**Method:** Timed-pregnant Sprague-Dawley rats were dosed with DBP at 500 mg/kg/day on gestation day (GD) 14 and 15, 15 and 16, 16 and 17, 17 and 18, 18 and 19, or 19 and 20 (GD 0=plug day). Anogenital distance (AGD) was measured | Carruthers and Foster, 2005b |

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| on postnatal day (PND) 1 and 13, while areloa number was recorded on PND 13 only. After weaning, males were allowed to mature to PND 90 at which time they were necropsied. Areloa number and AGD were recorded and testes, epididymides, seminal vesicles, prostate gland, kidneys, and liver weighed. Blood serum was collected and assayed for total testosterone concentration.<br>**Results:** There were no observable effects on litter size, sex ratio, serum testosterone concentration, or mortality of pups. Statistically significant permanent reductions in AGD were seen in males exposed prenatally to DBP on GD 15 and 16 or GD 18 and 19. On PND 13, areola were present in males exposed to DBP on GD 15 and 16, 16 and 17, 17 and 18, and 19 and 20. However, permanent retention occurred only in males after DBP exposure on GD 16 and 17. Exposure to DBP on only GD 17 and 18 elicited a reduction in epididymal weights; while exposure on only GD 16 and 17 caused a significant increase in the weights of the testes due to edema. In this study, epididymal and testicular malformations were most prevalent after exposure to DBP on any gestational day. Epididymal malformations, characterized by agenesis of various regions and small or flaccid testes were significantly increased in DBP-exposed males only on GD 16 and 17. | |
| **Type/Objective:** Fetal exposure of male rats to DBP induces reproductive disorders similar to those in human testicular dysgenesis syndrome (TDS), including infertility, cryptorchidism, focal "dysgenetic areas," and Sertoli cell-only tubules in the adult testis. A developmental toxicity study was performed in rats to evaluate end points affected by DBP action in rats in fetal and adult life that are relevant to human TDS, and to compare their dose sensitivity.<br>**Conclusion:** A NOAEL of 20 mg/kg/d was established with multiple effects on male reproductive organs at 100 mg/kg/day.<br>**Method:**, Pregnant rats were gavaged daily with corn oil (control) or with 4, 20, 100, or 500 mg/kg DBP. We examined adult end points of TDS (infertility, cryptorchidism) and indicators within the fetal testis of dysgenesis [abnormal Leydig cell (LC) aggregation, multinucleated gonocytes (MNGs)], as well as conditions that may result from these indicators in adulthood (occurrence of focal dysgenetic areas). Fetal testis weight and testicular testosterone levels were also evaluated.<br>**Results:** A dose dependent decrease in the fertility of the male offspring was noted starting at the 20 mg/kg dose when offspring were housed for one week with proven fertile females. At 500 mg/kg, infertility was statistically significant ($p=0.03$). Ninety percent of the animals exposed to 500mg/kg DBP showed cryptorchidism, the absence of one or both testes from the scrotum, and a significant decrease in testicular weight at gd 21.5 and adulthood. Testicular testosterone levels were significantly decreased in gd 21.5 animals with 100 and 500 mg/kg exposures ($p<0.05$ and $p<0.001$, respectively). In gd 21.5 testis sections, the authors noted an increase in occurrence of multinucleated gonocytes starting at 20 mg/kg, with significance achieved at 100 mg/kg ($p<0.001$), a decrease in Leydig cell number, and an increase in Leydig cell size at the 100 and 500 mg/kg doses. These fetal endpoints suggest abnormal development of the testis. Focal dysgenesis in adult rats was statistically significant at 500 mg/kg dose ($p=0.029$), although focal dysgenesis was also noted with the 100 mg/kg dose. Focal dysgenesis was defined as malformed seminiferous tubules with intratubular Leydig cells and immature sertoli cells in testis with no other malformations. The authors concluded that the fetal endpoints were the most sensitive to DBP effects. Since infertility and cryptorchidism were only | Mahood et al, 2007 (Results taken from summary in CPSC, 2010) |

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| significantly increased at the highest dose, they were insensitive end points for the use of investigating lower dose effects of DBP exposure in fetal life. | |
| **Type/Objective:** Devepomental toxicity study in rats was performed with a comparison of hypospadic (a malformation where the urethral opening is not at the top of the penis) and non-hypospadic male Sprague Dawley rats, 10 rats/group.<br>**Conclusion:** Rats showing hypospadia were more severely affected by DBP exposure than those rats not showing hypospadia from the same litter.<br>**Method:** Pregnant rats were dosed by gastric intubation with 0, 250, 500, 750, or 1000 mg/kg-day DBP from gd 14 to gd 18.<br>**Results:** Rats with hypospadia showed a decrease in liver, kidney, prostate, testes, and epididymis weight at 500 and 750 mg/kg-day when compared to non-hypospadic rats in the same dose group. The same organ weights of the nonhypospadiac rats were also significantly decreased compared to controls ($p<0.05$). In addition, adrenal and pituitary glands were statistically significantly ($p<0.05$) increased starting at 500 mg/kg-day in the hypospadic and non-hypospadic rats compared to controls. | Jiang et al, 2007, as summarized in CPSC, 2010 |
| **Type/Objective:** A **testicular dysgenesis**-like syndrome is induced in rats by fetal exposure to DBP. A key feature of this is the formation of focal dysgenetic areas comprising malformed seminiferous cords/tubules and intratubular Leydig cells (ITLC), but how and why these arise remains unclear. The present study was performed to investigate the<br>**Conclusion:** The present studies show that differentiation of the fetal Leydig cells is drastically delayed at e15.5 after DBP exposure, which may be indicative of a wider delay in testis cell development and organisation, and this might account for some of the unexplained findings.<br>**Method:** The present study has used combinations of cell-specific markers and immunohistochemistry.<br>**Results:** The results show that focal dysgenetic areas and ITLC first appear postnatally at 4-10 days of age, but this only occurs in treatment groups in which formation of fetal Leydig cell aggregation is induced between e17.5 and e21.5. Extreme variability in the formation and size of the Leydig cell aggregates probably accounts for the equally extreme variation in occurrence and size of focal dysgenetic areas postnatally. DBP-induced fetal Leydig cell aggregation traps Sertoli and other cells within the aggregates, but it is unclear why this happens nor why cords fail to form prenatally in these cell mixtures but do elsewhere in the fetal testis. | Hutchison et al, 2008 |
| **Type/Objective:** This developmental toxicity study was performed to determine whether in utero exposure to DIBP would induce permanent and dose-responsive alterations of male reproductive development. Groups of dams also received DBP for comparison.<br>**Conclusion:** Our results show that DIBP can cause severe and specific adverse effects on the male rat reproductive development, with a pattern similar to that of DBP. However, DIBP appeared slightly less potent than DBP in inducing malformations.<br>**Method:** Pregnant Sprague-Dawley rats were administered olive oil (vehicle control), DIBP or DBP, by gavage on gestation Days 12-21, at doses of 125, 250, 500, 625mgDIBP/(kg day) and 500mgDBP/(kg day).<br>**Results:** DIBP caused no overt maternal toxicity, nor reduced litter size. Male offspring displayed reduced neonatal anogenital distance (Postnatal day 1, PND) at 250mgDIBP/(kg day) and higher doses, and dose-related retention of | Saillenfait et al, 2008 |

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| areolas/nipples (PND 12-14). Preputial separation (onset of puberty) was delayed in male offspring at 500 and 625mgDIBP/(kg day). Hypospadias, cleft prepuce, and undescended testis were observed in males (11-12 or 16-17 weeks old) exposed in utero to 500 and 625mgDIBP/(kg day). Histopathological lesions were also present in adult testes, mainly consisting in seminiferous tubule degeneration. | |
| **Type/Objective:** Dietary developmental toxicity study in rats<br>**Conclusion:** Our results, when compared to previously conducted gavage studies, indicate that approximately equal doses of oral DBP exposure of pregnant rats, from diet or gavage, result in similar responses in male offspring.<br>**Method:** This study characterized the developmental toxicity of dietary DBP. Pregnant CD rats were given nominal doses of 0, 100, or 500 mg DBP/kg/day in diet (actual intake 0, 112, and 582 mg/kg/day) from gestational day (GD) 12 through the morning of GD 19. Rats were killed 4 or 24 hr thereafter.<br>**Results:** DBP dietary exposure resulted in significant dose-dependent reductions in testicular mRNA concentration of scavenger receptor class B, member 1; steroidogenic acute regulatory protein; cytochrome P450, family 11, subfamily a, polypeptide 1; and cytochrome P450 family 17, subfamily a, polypeptide 1. These effects were most pronounced 4 hr after the end of exposure. Testicular testosterone was reduced 24 hr post-exposure in both DBP dose groups and 4 hr after termination of the 500-mg DBP/kg/day exposure. Maternal exposure to 500 mg DBP/kg/day induced a significant reduction in male offspring's anogenital distance indicating in utero disruption of androgen function. Leydig cell aggregates, increased cord diameters, and multinucleated gonocytes were present in DBP-treated rats. Monobutyl phthalate, the developmentally toxic metabolite of DBP, and its glucuronide conjugate were found in maternal and fetal plasma, amniotic fluid, and maternal urine. | Struve et al, 2009 |
| **Type/Objective:** Androgens may be important regulators of Sertoli cell (SC) proliferation perinatally, with implications for the testicular dysgenesis syndrome (TDS) hypothesis. Fetal exposure of rats to 500 mg DBP/kg reduces fetal testosterone production and SC number at birth, but SC number recovers to normal by postnatal d (Pnd)25. It is unclear when and how SC proliferation is affected prenatally by DBP exposure or when and how postnatal compensation occurs. This study addressed these questions and investigated whether continued maternal exposure to DBP or to flutamide from Pnd1-Pnd15 could prevent SC number compensation, because this would have implications for how sperm counts might be lowered in TDS.<br>**Conclusion:** Our results provide further evidence that perinatal SC proliferation is androgen dependent and, importantly, show that similar exposure of mothers to antiandrogenic chemicals before birth and during lactation reduces final SC number, with implications for the origin of low sperm counts in TDS.<br>**Method:**<br>**Results:** DBP exposure attenuated SC proliferation by 7-18% throughout embryonic d (e)15.5-e21.5 ($P < 0.05$ at e21.5). After birth, SC proliferation increased significantly (>1.5-fold) between Pnd6 and Pnd10 in prenatally DBP-exposed animals, explaining the compensation. Continued maternal administration of DBP after birth attenuated (19% reduction) SC number compensation at Pnd25 and maternal administration of flutamide (100 mg/kg . d) to prenatally DBP-exposed animals was even more effective (42% reduction), suggesting the postnatal compensatory increase in SC proliferation after prenatal DBP exposure is androgen dependent. SC maturation (Pnd25) was unaffected, based on analysis of expression of key proteins, but lumen formation/expansion | Auharek at al, 2010 |

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| was attenuated in parallel with treatment-induced reduction in SC number. | |
| **Type/Objective:** The objectives of this study were to investigate the dysplasia, histological malformations, and genetic abnormalities in male rats induced by maternal exposure to DBP.<br>**Conclusion:** These results conclusively demonstrate for the first time that in utero exposure to DBP leads to an increased likelihood for the development of anorectal malformations (ARMs) and subsequent complicating megacolon in male rat offspring. Serum testosterone in males rats with ARMs was lower than controls, along with additional testosterone-related endpoints.<br>**Method:**<br>**Results:** The incidence of ARMs was 39.5% in male offspring and all abnormal pups were complicated with secondary megacolon. General images, histological analysis and anatomy examination confirmed the malformation. The development abnormalities such as decreased bodyweight (BW) and anogenital distance (AGD), shortened body lengths (with tail removed), as well as increased abdominal circumference were observed at different developmental stages of ARMs in male rat. The developmental abnormalities in both solid organs (brain, heart, liver, spleen, lung and kidney) and reproductive organs (testes and epididymis) of abnormal pubs on PND35 were also investigated. In addition, the serum testosterone (T) level of ARMs in male rats on PND1 was significantly lower than that of controls with accompanying reduced expression of androgen receptor (AR), sonic hedgehog (Shh) and bone morphogenetic protein 4 (Bmp4) mRNA from tissues of the terminal rectum. | Jiang et al, 2011 |
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| **Type/Objective:** A developmental toxicity study used p53-deficient mice due to their ability to display greater resistance to apoptosis during development. This model was chosen to determine whether multinucleated germ cells (MNG) induced by gestational DBP exposure could survive postnatally and evolve into testicular germ cell cancer.Pregnant dams were dosed with 500 mg DBP/kg/day on GD 12 to birth.<br>**Conclusion:** This unique model identified a role for p53 in the perinatal apoptosis of DBP-induced MNGs and provided insight into the long-term effects of gestational DBP exposure within a p53-null environment.<br>**Method:** Pregnant dams were exposed to DBP (500 mg/kg/day) by oral gavage from gestational day 12 until birth. Perinatal effects were assessed on gestational day 19 and postnatal days 1, 4, 7, and 10 for the number of MNGs present in control and DBP-treated p53-heterozygous and null animals.<br>**Results:** As expected, DBP exposure induced MNGs, with greater numbers found in p53-null mice. Additionally, there was a time-dependent decrease in the incidence of MNGs during the early postnatal period. Histologic examination of adult mice exposed in utero to DBP revealed persistence of abnormal germ cells only in DBP-treated p53-null mice, not in p53-heterozygous or wild-type mice. Immunohistochemical staining of perinatal MNGs and adult abnormal germ cells was negative for both octamer-binding protein 3/4 and placental alkaline phosphatase. | Saffarini et al, 2012 |
| **Type/Objective:** Little is known about the effects of DBP's metabolite, MBP, on preimplantation embryo development.<br>**Conclusion:** Together, the results indicate a possible relationship between MBP exposure and developmental failure in preimplantation embryos.<br>**Method:**<br>**Results:** Treatment of embryos with $10^{-3}$ M MBP impaired developmental | Chu et al, 2013 |

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| competency, whereas exposure to $10^{-4}$ M MBP delayed the progression of preimplantation embryos to the blastocyst stage. Furthermore, reactive oxygen species (ROS) levels in embryos were significantly increased following treatment with $10^{-3}$ M MBP. In addition, $10^{-3}$ M MBP increased apoptosis via the release of cytochrome c, whereas immunofluorescent analysis revealed that exposure of preimplantation embryos to MBP concentration-dependently ($10^{-5}$, $10^{-4}$ and $10^{-3}$ M) decreased DNA methylation. | |
| **Type/Objective:** Exposure in children to DBP has been thought to be one of the reasons causing a trend of advanced pubertal timing in girls. Puberty starts from hypothalamic gonadotropin-releasing hormone release which is controlled by many factors including neurotransmitter kisspeptin and its receptor GPR54. These neural organization or reorganization happens in hypothalamus during neonatal or prepubertal period which may be two target windows of DBP exposure.<br>**Conclusion:** These results demonstrated small dose of DBP could induce earlier pubertal timing in females and both neonatal and prepubertal periods were critical windows for DBP exposure.<br>**Method:** The present study was designed to determine: (1) the difference between the effects of neonatal and prepubertal DBP exposure on female pubertal timing; (2) whether kisspeptin/GPR54 expression in hypothalamus would respond to neonatal and prepubertal DBP exposure differently. Female Sprague-Dawley rats were exposed by subcutaneous injection of 0.5, 5 and 50mg/kg DBP during postnatal day (PND)1-5 (neonatal) or PND 26-30 (prepubertal). Physiological data demonstrated that both neonatal and prepubertal DBP exposure could advance pubertal timing significantly accompanied by irregular estrous cycles but only a little gonadal impairment.<br>**Results:** Exposure-period-related difference was found significant with prepubertal exposure groups having longer estrous cycle duration, heavier at vaginal opening and having higher serum estradiol level compared with neonatal exposure groups. Molecular data showed an up-regulated trend in kisspeptin mRNA and immunoreactivity levels of hypothalamic area arcuate but a down-regulation in GPR54 mRNA expression after P1-5 DBP treatment. In P26-30 groups, kisspeptin mRNA and immunoreactivity levels tended to be lower after DBP treatment. | Hu et al, 2013 |
| **Type/Objective:** To investigate whether such early gestational and/or lactational exposure can influence the later adult-type Leydig cell phenotype.<br>**Conclusion:** These results support the notion that maternal exposure to certain xenobiotics can also influence the development of the adult-type Leydig cell population, possibly through an effect on the Leydig stem cell population.<br>**Method:** Sprague-Dawley rats were exposed to DBP from GD 14.5 to PND 6 or diethylstilbestrol (DES; from GD14.5 to GD16.5) during a short gestational/lactational window, and male offspring subsequently analysed for various postnatal testicular parameters.<br>**Results:** All offspring remained in good health throughout the study. Maternal xenobiotic treatment appeared to modify specific Leydig cell gene expression in male offspring, particularly during the dynamic phase of mid-puberty, with serum INSL3 concentrations showing that these compounds led to a faster attainment of peak values, and a modest acceleration of the pubertal trajectory. Part of this effect appeared to be due to a treatment-specific impact on Leydig cell proliferation during puberty for both xenobiotics. | Ivell et al, 2013 |
| **Type/Objective:** To evaluate Leydig cells<br>**Conclusion:** Atypical Leydig cell (LC) hyperplasia was seen in 20-week-old rats | Wakui et al, 2013 |

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| with low testosterone and high luteinizing hormone levels after treatment of mothers with DBP during gestation.<br>**Method:** Pregnant Sprague-Dawley rats received 100 mg DBP/kg/day on GD 12 to 21 and male offspring were evaluated for effects on LCs.<br>**Results:** Light microscopy revealed LC hyperplasia surrounded by severely degenerated seminiferous tubules. Aggregated LCs had large ovoid nuclei with nucleoli and abundant eosinophilic cytoplasm. Immunohistochemical analysis showed expression of proliferating cell nuclear antigen and vimentin in many hyperplastic LCs. Electron microscopy revealed atypical nuclei, abundant free ribosomes, stripped rough endoplasmic reticulum, intermediate-size filaments, elongated cytoplasmic filopodia, atypical tight junctions, and cilia formations, but smooth endoplasmic reticulum was scarcely observed. | |
| **Type/Objective:** To isolate and identify differentially expressed proteins in testis of rat fetuses after maternal exposure to DBP.<br>**Conclusion:** The present study had found several differentially regulated proteins and demonstrated the differential expression of Prdx6, AnxA5 and UchL1 in fetal rat testis after maternal exposure to DBP, when compared with controls. Combining the cellular location of these proteins and their function in other tissues, the results of this study indicated that oxidative injury and abnormal apoptotic regulation might participate the formation of testicular dysgenesis in fetuses of dams exposed to DBP.<br>**Method:** Pregnant rats were daily treated by gavage with 1 ml/kg corn oil or 750 mg/kg DBP from GD14 to GD18. We used the technique of proteomic analysis to compare the testis protein patterns obtained by two-dimensional gel electrophoresis from fetal rats of gestation day 19.<br>**Results:** We found significant differences in protein spot intensities compared to control. Subsequently several of these variant protein spots were identified by mass spectrometry. Peroxiredoxin 6 (Prdx6), annexin A5 (AnxA5) and ubiquitin carboxyl-terminal hydrolase isozyme L1 (UchL1) were three of them, the differential expression of which were confirmed by western blotting. Further, immunohistochemical analyses of fetal rat testes sections were made to determine the cellular distribution of these proteins, consequently strong Prdx6 and AnxA5 stainings were found primarily in Leydig cells, while a weak UchL1 staining was found primarily in spermatogonium. | Shen H et al, 2013 |
| **Type/Objective:** Environmental compounds are known to promote epigenetic transgenerational inheritance of adult onset disease in subsequent generations (F1-F3) following ancestral exposure during fetal gonadal sex determination. The current study was designed to determine if a mixture of plastic derived endocrine disruptor compounds bisphenol-A (BPA), bis(2-ethylhexyl)phthalate (DEHP) and dibutyl phthalate (DBP) at two different doses promoted epigenetic transgenerational inheritance of adult onset disease and associated DNA methylation epimutations in sperm.<br>**Conclusion:** Observations demonstrate that a mixture of plastic derived compounds, BPA and phthalates, can promote epigenetic transgenerational inheritance of adult onset disease. The sperm DMR provide potential epigenetic biomarkers for transgenerational disease and/or ancestral environmental exposures.<br>**Method:** Gestating F0 generation females were exposed to either the "plastics" or "lower dose plastics" mixture during embryonic days 8 to 14 of gonadal sex determination and the incidence of adult onset disease was evaluated in F1 and F3 generation rats. | Manikkam et al, 2013 |

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| **Results:** There were significant increases in the incidence of total disease/abnormalities in F1 and F3 generation male and female animals from plastics lineages. Pubertal abnormalities, testis disease, obesity, and ovarian disease (primary ovarian insufficiency and polycystic ovaries) were increased in the F3 generation animals. Kidney and prostate disease were only observed in the direct fetally exposed F1 generation plastic lineage animals. Analysis of the plastics lineage F3 generation sperm epigenome previously identified 197 differential DNA methylation regions (DMR) in gene promoters, termed epimutations. A number of these transgenerational DMR form a unique direct connection gene network and have previously been shown to correlate with the pathologies identified. | |
| **Type/Objective:** Previous analysis of in utero DBP-exposed fetal rat testes indicated that DBP's antiandrogenic effects were mediated, in part, by indirect inhibition of steroidogenic factor 1 (SF1), suggesting that peroxisome proliferator-activated receptor alpha (PPARα) might be involved through coactivator (CREB-binding protein [CBP]) sequestration.<br>**Conclusion:** The data indicate that PPARα may act as an indirect transrepressor of SF1 on steroidogenic genes in fetal rat testes in response to DBP treatment.<br>**Method:** To test this hypothesis, we have performed chromatin immunoprecipitation (ChIP) microarray analysis to assess the DNA binding of PPARα, SF1, CBP, and RNA polymerase II in DBP-induced testicular maldevelopment target genes.<br>**Results:** Pathway analysis of expression array data in fetal rat testes examined at gestational day (GD) 15, 17, or 19 indicated that lipid metabolism genes regulated by SF1 and PPARα, respectively, were overrepresented, and the time dependency of changes to PPARα-regulated lipid metabolism genes correlated with DBP-mediated repression of SF1-regulated steroidogenesis genes. ChIP microarrays were used to investigate whether DBP-mediated repression of SF1-regulated genes was associated with changes in SF1 binding to genes involved in DBP-induced testicular maldevelopment. DBP treatment caused reductions in SF1 binding in CYP11a, StAR, and CYP17a. Follicle-stimulating hormone receptor (FSHR), regulated by SF1 but unaffected by DBP-treatment, also contained SF1-binding peaks, but DBP did not change this compared with control. GD15 and GD19 fetal testes contained PPARα protein-binding peaks in CYP11a, StAR, and CYP17a regulatory regions. In contrast to its repressive effect on SF1, DBP treatment caused increases in these peaks compared with control. PPARα-binding peaks in the FSHR promoter were not detected in GD15 samples. Hence, the repressive effect of DBP on SF1-regulated steroidogenic genes correlates with inhibition of SF1-DNA binding and increased PPARα-DNA binding. | Plummer et al, 2013 |
| **Type/Objective:** An approach for evaluating and integrating genomic data in chemical risk assessment was developed based on the lessons learned from performing a case study for DBP.<br>**Conclusion:** A general approach for integrating genomic data in chemical assessment was developed<br>**Method:** A case study prototype approach was first developed in accordance with EPA guidance and recommendations of the scientific community. DBP was selected for the case study exercise. The scoping phase of the DBP case study was conducted by considering the available DBP genomic data, taken together with the entire data set, for whether they could inform various risk assessment aspects, such as toxicodynamics, toxicokinetics, and dose-response. A description of weighing the available DBP data set for utility in risk assessment provides an | Euling et al, 2013a |

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| example for considering genomic data for future chemical assessments. As a result of conducting the scoping process, two questions--Do the DBP toxicogenomic data inform 1) the mechanisms or modes of action?, and 2) the interspecies differences in toxicodynamics?--were selected to focus the case study exercise. Principles of the general approach include considering the genomics data in conjunction with all other data to determine their ability to inform the various qualitative and/or quantitative aspects of risk assessment, and evaluating the relationship between the available genomic and toxicity outcome data with respect to study comparability and phenotypic anchoring.<br>**Results:** Based on experience from the DBP case study, recommendations and a general approach for integrating genomic data in chemical assessment were developed to advance the broader effort to utilize 21st century data in risk assessment. | |
| **Type/Objective:** An evaluation of the toxicogenomic data set for DBP and male reproductive developmental effects was performed as part of a larger case study to test an approach for incorporating genomic data in risk assessment.<br>**Conclusion:** This case study on DBP identified data gaps and research needs for the use of toxicogenomic data in risk assessment. Furthermore, this study demonstrated an approach for evaluating toxicogenomic data in human health risk assessment that could be applied to future chemicals.<br>**Method:** The DBP toxicogenomic data set is composed of nine in vivo studies from the published literature that exposed rats to DBP during gestation and evaluated gene expression changes in testes or Wolffian ducts of male fetuses. The exercise focused on qualitative evaluation, based on a lack of available dose-response data, of the DBP toxicogenomic data set to postulate modes and mechanisms of action for the male reproductive developmental outcomes, which occur in the lower dose range. A weight-of-evidence evaluation was performed on the eight DBP toxicogenomic studies of the rat testis at the gene and pathway levels.<br>**Results:** The results showed relatively strong evidence of DBP-induced downregulation of genes in the steroidogenesis pathway and lipid/sterol/cholesterol transport pathway as well as effects on immediate early gene/growth/differentiation, transcription, peroxisome proliferator-activated receptor signaling and apoptosis pathways in the testis. Since two established modes of action (MOAs), reduced fetal testicular testosterone production and Insl3 gene expression, explain some but not all of the testis effects observed in rats after in utero DBP exposure, other MOAs are likely to be operative. A reanalysis of one DBP microarray study identified additional pathways within cell signaling, metabolism, hormone, disease, and cell adhesion biological processes. These putative new pathways may be associated with DBP effects on the testes that are currently unexplained. | Euling et al, 2013b |
| **Type/Objective:** The study was conducted to assess the effects of in utero DBP and butyl benzyl phthalate (BBP) exposure during late gestation on offspring's development and reproductive system of male rats.<br>**Conclusion:** The data suggests that DBP and BBP exposure during late gestation period might have adverse effects on offspring's development, spermatogenesis, and steroidogenesis in adult rats.<br>**Method:** Pregnant rats were treated orally with DBP (2, 10, 50 mg/kg), BBP (4, 20, 100 mg/kg), and diethylstilbestrol (DES) 6 µg/kg (positive control) from GD14 to parturition.<br>**Results:** A significant reduction in dams' body weight on GD21 in DBP-, BBP-, | Ahmad et al, 2014 |

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| and DES-treated groups was observed. The gestation length was considerably elevated in the treated groups. Decline in male pups' body weight was significant at PND75 in DBP- (50 mg/kg), BBP- (20,100 mg/kg), and DES-treated groups. The weight of most of the reproductive organs and sperm quality parameters was impaired significantly in DBP- (50 mg/kg) and BBP- (100 mg/kg) treated groups. Further, a non-significant decline in testicular spermatid count and daily sperm production was also monitored in treated groups. A significant reduction in serum testosterone level in BBP (100 mg/kg), whereas the testicular activity of 17β-HSD was declined non-significantly in the treated groups with respect to control. | |
| **Type/Objective:** This study was designed to investigate the reproductive health in adult male rats exposed to DBP during embryonic development.<br>**Conclusion:** Transplacental exposure to DBP impaired male reproductive performance by decreasing steroidogenesis and spermatogenesis.<br>**Method:** Pregnant rats were injected with DBP [dose and route were not given in abstract] and F1 male rats were weaned and on postnatal day 100, used for mating with normal cycling females to assess reproductive performance. After completion of cohabitation period, rats were analyzed for other reproductive end points.<br>**Results:** Transplacental exposure to DBP significantly decreased fertility in adult male rats. Prenatal exposure to DBP significantly decreased sperm density, number of motile sperms, viable sperms, and hypoosmotic swelling tail coiled sperms with an increase in morphological abnormalities in sperms. Testicular steroidogenic enzyme activity levels and serum testosterone levels were significantly decreased in rats exposed to DBP during embryonic development. | Giribabu et al, 2014 |
| **Type/Objective:** Mounting evidence has indicated the crucial role of Wnt5a in the embryonic development including guts. However, the Wnt5a involvement in the process of anorectal malformations (ARMs) remains unclear.<br>**Conclusion:** Results demonstrate the aberrant expression of Wnt5a during anorectal development, which suggests that Wnt5a might be involved in DBP-induced ARMs.<br>**Method:** In this study, we examined the expression of Wnt5a during ARMs development in the offspring of DBP-treated pregnant rats. During the neonatal period, Wnt5a expression was evaluated in the terminal rectum of ARM offspring, non-ARM littermates and controls<br>**Results:** Using real-time polymerase chain reaction (real-time PCR), western-blot analysis and immunohistochemistry approaches, we found a significant decrease of Wnt5a expression in DBP-induced ARMs rats. | Li EH et al, 2014 |
| **Type/Objective:** This study was designed to explore the effect of environmental endocrine disruptors (EEDs) on sexual differentiation in androgen receptor (AR)-/-, AR+/- and AR+/+ male mice by using a Cre-loxP conditional knockout strategy to generate AR knockout mice.<br>**Conclusion:** Exposure to EEDs induces hypospadias in heterozygous and wild-type male mice offspring during sexual differentiation, but has no effect on homozygous offspring. Therefore, EEDs play an important role during the third stage of sexual differentiation.<br>**Method:** By mating flox-AR female mice with AR-Cre male mice, the offspring male mice which were produced were examined. Mice not subjected to any type of intervention were used as the controls. Furthermore, male mice of different genotypes were selected and further divided into subgroups as follows: the control group, bisphenol A (BPA) group and the dibutyl phthalate (DBP) group. The expression of the Wilms tumor 1 (WT1), lutropin/choriogonadotropin receptor | Liu D et al, 2015 |

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| (LHR), 17-β-hydroxysteroid dehydrogenase type 3 (17βHSD3) and steroid-5-alpha-reductase, alpha polypeptide 2 (SRD5A2) genes was determined by RT-qPCR and western blot analysis.<br>**Results:** There was no statistically significant difference in the weight of the mice between the control group and the knockout group (P>0.05). The results revealed that, compared with the control group, in the knockout group, anogenital distance was shortened, and testicular weight and testosterone levels were decreased; estradiol levels were elevated; the differences were statistically significant (P<0.05). In the group of AR+/- male mice exposed to 100 mg/l EEDs, hypospadias was successfully induced, suggesting that EEDs are involved in the embryonic stage of sexual development in male mice. The quantitative detection of WT1, LHR, 17βHSD3 and SRD5A2 gene expression by RT-qPCR and western blot analysis indicated that these genes were significantly downregulated in the mice in the BPA group. | |
| **Type/Objective:** DBP causes masculinization disorders in rats, raising concern for similar effects in humans. We investigated whether DBP exposure impairs steroidogenesis by the human fetal testis. The aim of the study was to determine effects of DBP exposure on testosterone production by normally growing human fetal testis xenografts.<br>**Conclusion:** Exposure of human fetal testes to DBP is unlikely to impair testosterone production as it does in rats.<br>**Method:** Human fetal testes (14-20 wk gestation; n=12) were xenografted into castrate male nude mice that were treated for 4-21 d with vehicle, or 500 mg/kg·d DBP, or monobutyl phthalate (active metabolite of DBP); all mice were treated with human chorionic gonadotropin to mimic normal human pregnancy. Rat fetal testis xenografts were exposed for 4 d to DBP as a positive control. Testosterone production was assessed by measuring host serum testosterone and seminal vesicle (SV) weights at termination, plus testis gene expression (rats).<br>**Results:** Human fetal testis xenografts showed similar survival (~80%) and total graft weight (8.6 vs. 10.1 mg) in vehicle and DBP-exposed hosts, respectively. Serum testosterone (0.56 vs. 0.64 ng/ml; P>0.05) and SV weight (67.2 vs. 81.9 mg; P>0.05) also did not differ. Exposure to monobutyl phthalate gave similar results. In contrast, exposure of rat fetal xenografts to DBP significantly reduced SV weight and testis Cyp11a1/StAR mRNA expression and lowered testosterone levels, confirming that DBP exposure can inhibit steroidogenesis in xenografts, further validating the negative findings on testosterone production in the human. | Mitchell et al, 2012 |

**BACKGROUND INFORMATION FOR TABLE 36B: HUMAN DATA ON ADVERSE EFFECTS ON DEVELOPMENT OF THE OFFSPRING**

| Summary of Study | Reference |
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| **Type/Objective:** The objective in this study was to assess the effect of occupational exposure to high levels of phthalate esters on the balance of gonadotropin and gonadal hormones including luteinizing hormone, follicle-stimulating hormone, free testosterone (fT), and estradiol.<br>**Conclusion:** A modest and significant reduction of serum fT was observed in workers with higher levels of urinary MBP and MEHP compared with unexposed workers.<br>**Method:** We examined urine and blood samples of 74 male workers at a factory producing unfoamed polyvinyl chloride flooring exposed to DBP and DEHP and compared them with samples from 63 male workers from a construction company, group matched for age and smoking status.<br>**Results:** Compared to the unexposed workers, the exposed workers had substantially and significantly elevated concentrations of mono-n-butyl phthalate (MBP; 644.3 vs. 129.6 microg/g creatinine, $p < 0.001$) and mono-2-ethylhexyl phthalate (MEHP; 565.7 vs. 5.7 microg/g creatinine, $p < 0.001$). fT was significantly lower (8.4 vs. 9.7 microg/g creatinine, $p = 0.019$) in exposed workers than in unexposed workers. fT was negatively correlated to MBP ($r = -0.25$, $p = 0.03$) and MEHP ($r = -0.19$, $p = 0.095$) in the exposed worker group. Regression analyses revealed that fT decreases significantly with increasing total phthalate ester score (the sum of quartiles of MBP and MEHP; $r = -0.26$, $p = 0.002$). | Pan et al, 2006 |
| **Type/Objective:** To assess play behaviour in relation to phthalate metabolite concentration in prenatal urine samples, we recontacted participants in the Study for Future Families whose phthalate metabolites had been measured in mid-pregnancy urine samples.<br>**Conclusion:** These data, although based on a small sample, suggest that prenatal exposure to antiandrogenic phthalates may be associated with less male-typical play behaviour in boys. The findings suggest that these ubiquitous environmental chemicals have the potential to alter androgen-responsive brain development in humans.<br>**Method:** Mothers completed a questionnaire including the Pre-School Activities Inventory, a validated instrument used to assess sexually dimorphic play behaviour. We examined play behaviour scores (masculine, feminine and composite) in relationship to (log(10)) phthalate metabolite concentrations in mother's urine separately for boys ($N = 74$) and girls ($N = 71$). Covariates (child's age, mother's age and education and parental attitude towards atypical play choices) were controlled using multivariate regression models.<br>**Results:** Concentrations of dibutyl phthalate metabolites, mono-n-butyl phthalate (MnBP) and mono-isobutyl phthalate (MiBP) and their sum, were associated with a decreased (less masculine) composite score in boys (regression coefficients - | Swan et al, 2010 |

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| 4.53,-3.61 and -4.20, p = 0.01, 0.07 and 0.04 for MnBP, MiBP and their sum respectively). Concentrations of two urinary metabolites of di(2-ethylhexyl) phthalate (DEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and the sum of these DEHP metabolites plus mono(2-ethylhexyl) phthalate were associated with a decreased masculine score (regression coefficients -3.29,-2.94 and -3.18, p = 0.02, 0.04 and 0.04) for MEHHP, MEOHP and the sum respectively. No strong associations were seen between behaviour and urinary concentrations of any other phthalate metabolites in boys, or between girls' scores and any metabolites. | |
| **Type/Objective:** The goal was to explore the association between prenatal di(2-ethylhexyl) phthalate and dibutyl phthalate exposure and the Mental and Psychomotor Developmental Indices (MDI and PDI, respectively) of the Bayley Scales of Infant Development at 6 months.<br>**Conclusion:** The results suggest that prenatal exposure to phthalates, including DBP, may be inversely associated with the MDI and PDI of infants, particularly males, at 6 months.<br>**Method:** Between 2006 and 2009, 460 mother-infant pairs from Seoul, Cheonan, and Ulsan, Korea, participated. Prenatal mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-n-butyl phthalate (MBP) were measured in one urine sample acquired from each mother during the third trimester of pregnancy. Associations with log-transformed creatinine-corrected phthalate concentrations were estimated using linear regression models adjusted for potential confounders.<br>**Results:** MDI was inversely associated with the natural log concentrations (micrograms per gram creatinine) of MEHHP [β = -0.97; confidence interval (CI), -1.85 to -0.08] and MEOHP (β = -0.95; CI, -1.87 to -0.03), and PDI was inversely associated with MEHHP (β = -1.20; CI, -2.33 to -0.08). In males, MDI was inversely associated with MEHHP (β = -1.46; CI, -2.70 to -0.22), MEOHP (β = -1.57; CI, -2.87 to -0.28), and MBP (β = -0.93; CI, -1.82 to -0.05); PDI was inversely associated with MEHHP (β = -2.36; CI, -3.94 to -0.79), MEOHP (β = -2.05; CI, -3.71 to -0.39), and MBP (β = -1.25; CI, -2.40 to -0.11). No significant linear associations were observed for females. | Kim Y et al, 2011 |
| **Type/Objective:** Hypospadias is a birth defect found in boys in which the urinary tract opening is not at the tip of the penis. The etiology of hypospadias is still unidentified, but endocrine disruptors are considered as one possible cause of hypospadias. In this study, levels of specific endocrine disruptors, including DBP, were measured in blood and urine of mothers.<br>**Conclusion:** No relation between the levels of endocrine disrupters and hypospadias was found. [Sample size was not in the abstract used for this summary.]<br>**Method:** The target compounds included 5 phthalates DEHP, DBP, MEHP, MBP and phthalic acid (PA), 2 alkylphenols (n-nonylphenol (n-NP) and t-octylphenol (t-OP)) and bisphenol A. The association between these 8 endocrine disruptors and hypospadias was studied. The levels of endocrine disruptors in the urine and plasma of a control group were compared with those of a patient group.<br>**Results:** DEHP (P = 0.006) and n-NP (P = 7.26e-6) in the urine samples and PA (P = 0.009) and BPA (P = 7.22e-10) in the plasma samples showed a significant association with hypospadias. The levels of endocrine disruptors in the urine and plasma of the mothers were also compared to those of the patients to investigate the metastasis of the endocrine disruptors from the mother. These levels did not, however, show a relationship with hypospadias (R(2) = 0.001-0.563). | Choi et al, 2012 |

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| **Type/Objective:** To assess the relationship between prenatal exposure to phthalate esters and behavior syndromes in children at 8 years of age.<br>**Conclusion:** Our findings suggest positive associations between maternal DEHP and DBP exposure and externalizing domain behavior problems in 8-year-old children.<br>**Method:** A total of 122 mother-child pairs from the general population in central Taiwan were studied from 2000 to 2009. Mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-**butyl phthalate** (MBP), mono-benzyl phthalate (MBzP), and three di-(2-ethylhexyl) phthalate (DEHP) metabolites-mono-2-ethylhexyl, mono-2-ethyl-5-hydroxyhexyl, and mono-2-ethyl-5-oxohexyl phthalates (MEHP, MEHHP, and MEOHP)--were measured in maternal urine collected during the third trimester of pregnancy using liquid chromatography-electrospray ionization-tandem mass spectrometry. Behavioral syndromes of children at 8 years of age were evaluated using the Child Behavior Checklist (CBCL). Associations between log10-transformed creatinine-corrected phthalate concentrations and standardized scores of the CBCL were estimated using linear regression models or multinomial logistic regressions with adjustments for potential confounders.<br>**Results:** Externalizing problem scores were significantly higher in association with a 1-unit increase in log10-transformed creatinine-corrected concentrations of maternal MBP (β = 4.29; 95% CI: 0.59, 7.99), MEOHP (β = 3.74; 95% CI: 1.33, 6.15), and MEHP (β = 4.28 ; 95% CI: 0.03, 8.26) after adjusting for the child's sex, intelligence, and family income. Meanwhile, MBP and MEOHP were significantly associated with Delinquent Behavior and Aggressive Behavior scores. The same pattern was found for borderline and/or clinical ranges. | Lien et al, 2015 |

**BACKGROUND INFORMATION FOR TABLE 36C: OTHER STUDIES RELEVANT FOR ADVERSE EFFECTS ON DEVELOPMENT OF THE OFFSPRING**

| Summary of Study | Reference |
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| **Type/Objective:** In a previous studies, butyl benzyl phthalate (BBP) and DBP were found to be teratogenic when administered to rats on days 7-9 and days 13-15 pregnancy but not days 10-12. The present study was conducted to determine the phase specificity of the developmental toxicity of mono-n-**butyl phthalate** (MBuP) and to assess the role of MBuP in the developmental toxicity of BBP and DBP in rats.<br>**Conclusion:** These findings suggest that MBuP and/or its further metabolites may be responsible for the production of the developmental toxicity of BBP and DBP.<br>**Method:** Pregnant rats were given MBuP by gastric intubation at a dose of 500, 625 or 750 mg/kg on days 7-9, days 10-12 or days 13-15 of pregnancy.<br>**Results:** A significant increase in embryolethality was noted in pregnant rats given MBuP regardless of the days of treatment. No evidence of teratogenicity was found when MBuP was given on days 10-12. A significantly increased incidence of fetuses with external malformations was found after treatment with MBuP on days 7-9 and days 13-15 at 625 and 750 mg/kg. A significantly increased incidence of fetuses with skeletal malformations was observed after treatment with MBuP on days 7-9 at 500 mg/kg and above and on days 13-15 at 625 mg/kg and above. Deformity of the cervical vertebrae was predominantly observed after treatment with MBuP on days 7-9. Cleft palate and fusion of the sternebrae were exclusively found after treatment with MBuP on days 13-15. The dependence of gestational days of treatment on the manifestation of the developmental toxicity and the spectrum of fetal malformations induced by MBuP were consistent with those induced by BBP and DBP. | Ema et al, 1995 |
| **Type/Objective:** To further characterize the developmental toxicity of mono-n-**butyl phthalate** (MBuP), which is one of the major metabolites of n-butyl benzyl phthalate (BBP) and DBP.<br>**Conclusion:** Findings were dependent on doase and timing of those those doses.<br>**Method:** Pregnant rats were given MBuP by gastric intubation at a dose of 500, 625 or 750 mg/kg on days 7-9, days 10-12, or days 13-15 of pregnancy.<br>**Results:** A significantly increased incidence of postimplantation loss was noted in pregnant rats given MBuP on days 7-9 and days 10-12 at doses of 625 mg/kg and above and on days 13-15 at doses of 500 mg/kg and above. No evidence of teratogenicity was found when MBuP was given on days 10-12 of pregnancy. A significantly increased incidence of fetuses with external malformations was found after treatment with MBuP on days 7-9 and days 13-15 at doses of 625 and 750 mg/kg. A significantly increased incidence of fetuses with skeletal malformations was observed after treatment with MBuP on days 7-9 at doses of 500 mg/kg and above. | Ema et al, 1996 |

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| **Type/Objective:** Embryotoxic profiles of DBP and MBP were compared at midgestation.<br>**Conclusion:** These results provide strong evidence that DBP-induced embryotoxicity is mediated through its main metabolite MBP.<br>**Method:** Pregnant Sprague-Dawley rats were given a single oral dose of 1.8, 3.6, 5.4, or 7.2 mmol DBP or MBP/kg on GD 10. The embryos were examined for growth and development on GD 12, a window for observing the origin of the lethality previously reported at term after DBP administration on GD 10 (J. Appl. Toxicol., 1997, 17, 223-229).<br>**Results:** No increase in embryo lethality was observed within 48 hr after administration of DBP or MBP at doses up to 7.2 mmol/kg. Both DBP and MBP produced growth retardation and dysmorphogenesis in a dose-dependent manner. DBP and MBP were essentially without effects at 1.8 mmol/kg. Adverse effects appeared at 3.6 mmol DBP or MBP/kg. At 5.4 mmol DBP or MBP/kg, all the parameters of growth and development assessed (i.e. crown-rump length, head lengths, and number of somite pairs) were reduced, and 80-88% of the embryos were malformed. The spectrum of malformations observed with MBP closely resembled that produced by the parent compound. The commonest morphological alterations were those of the anterior part of the head, and involved the prosencephalon, the optic and otic systems, and the mandibular arches. In addition, maternal plasma was analyzed by HPLC for metabolic species after administration of 5.4 mmol (14C)DBP/kg. MBP accounted for most of the DBP-derived 14C, whereas DBP was barely detectable. | Langonne et al, 1998 |
| **Type/Objective:** To investigate estrogenic activity of DBP<br>**Conclusion:** Estrogenic activity of DBP was weak in in vitro assays and not observed in an in vivo assay.<br>**Method and Results:** Using an estrogen receptor (ER) competitive ligand binding assay, and mammalian and yeast based gene expression assays, the authors showed that DBP weakly competed with estradiol (E2) for the ER. DBP also showed 37% activity in a transiently transfected MCF-7 Gal-4 human ER construct at 10μM, where E2 is 100% at 10nM. DBP did not show any estrogenic activity *in vivo* when uterine wet weights and vaginal cell conification of ovariectomized Sprague Dawley rats orally treated with 20, 200, or 2000 mg/kg DBP dose were assessed. | Zacharewski et al, 1998, as summarized in CPSC, 2010 |
| **Type/Objective:** DBP might alter reproductive development by a different mechanism of action than flutamide or vinclozolin (V), which are AR antagonists, because the male offsprings display an unusually high incidence of testicular and epididymal alterations--effects rarely seen after in utero flutamide or V treatment. Here the authors presented original data describing the reproductive effects of 10 known or suspected anti-androgens<br>**Conclusion:** The in vivo data suggest that the chemicals we studied alter male sexual differentiation via different mechanisms. The anti-androgens V, P, and p,p'-DDE produce flutamide-like profiles that are distinct from those seen with DBP, DEHP, and L. The effects of PCB 169 bear little resemblance to those of any known anti-androgen.<br>**Method:** In this study, authors present data describing the reproductive effects of 10 known or suspected anti-androgens, including a Leydig cell toxicant ethane dimethane sulphonate (EDS, 50 mg kg-1 day-1), linuron (L, 100 mg kg-1 day-1), p,p'-DDE (100 mg kg-1 day-1), ketoconazole (12-50 mg kg-1 day-1), procymidone (P, 100 mg kg-1 day-1), chlozolinate (100 mg kg-1 day-1), iprodione (100 mg kg-1 day-1), DBP (500 mg kg-1 day-1), diethylhexyl phthalate | Gray et al, 1999 |

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| (DEHP, 750 mg kg-1 day-1), and polychlorinated biphenyl (PCB) congener no. 169 (single dose of 1.8 mg kg-1).<br>**Results:** Our analysis indicates that the chemicals discussed here can be clustered into three or four separate groups, based on the resulting profiles of reproductive effects. Vinclozolin, P, and DDE, known AR ligands, produce similar profiles of toxicity. However, p,p'-DDE is less potent in this regard. DBP and DEHP produce a profile distinct from the above AR ligands. Male offsprings display a higher incidence of epididymal and testicular lesions than generally seen with flutamide, P, or V even at high dosage levels. Linuron treatment induced a level of external effects consistent with its low affinity for AR [reduced anogenital distance (AGD), retained nipples, and a low incidence of hypospadias]. However, L treatment also induced an unanticipated degree of malformed epididymides and testis atrophy. In fact, the profile of effects induced by L was similar to that seen with DBP. These results suggest that L may display several mechanisms of endocrine toxicity, one of which involves AR binding. Chlozolinate and iprodione did not produce any signs of maternal or fetal endocrine toxicity at 100 mg kg-1 day-1. EDS produced severe maternal toxicity and a 45% reduction in size at birth, which resulted in the death of all neonates by 5 days of age. However, EDS only reduced AGD in male pups by 15%. Ketoconazole did not demasculinize or feminize males but rather displayed anti-hormonal activities, apparently by inhibiting ovarian hormone synthesis, which resulted in delayed delivery and whole litter loss. . | |
| **Type/Objective:** Embryotoxicity/teratogenicity of DBP and MBP<br>**Conclusion:**<br>**Method:** DBP and mono-n-butyl phthalate were each given separately once orally to pregnant Sprague-Dawley rats on GD 10 at 1.8, 3.6, 5.4, or 7.2 mmol/kg. Fetal growth and development evaluated on GD 12.<br>**Results:** Dose-related reduced growth and malformations occurred with both chemicals at $\geq$3.6 mmol/kg. Malformations commonly involved prosencephalon, optic nerve, and mandibular and maxillary processes. Types of effects and potency were approximately equivalent between DBP and its major metabolite. Effects with MBP were reproduced in 48-hr cultures of embryos exposed in vitro, showing direct action. | Saillenfait, et al, 2001 |
| **Type/Objective:** The objective of this study was to determine the chronology of lesion development by assessing the male reproductive tracts of rats exposed to DBP in utero.<br>**Conclusion:** These observations support the conclusion that DBP has primary effects on the testes, which are further compounded by increased testicular intratubular pressure resulting from malformations of the epididymides.<br>**Method:** Pregnant Sprague-Dawley rats were dosed by gavage on gestation days (GD) 12 to 21 with vehicle or 500 mg/kg/day of DBP. Fetuses were examined on GD 18 to 21 and male pups were necropsied on postnatal days (PND) 3, 7, 16, 21, 45 and 70.<br>**Results:** Gross lesions were not detectable during gestation or before PND 16. However, complete and partial absence of epididymides and vasa deferentia was observed on PND 16 and on later time points in combination with small, flaccid testes on PND 45 and 70. Histologically, aggregates of Leydig cells were seen in the testes on DBP-exposed fetuses that remained from GD 18 until PND 21. Multinucleated gonocytes were present within the seminiferous cords on GD 18 to 21 and were observed in reduced numbers on PND 3 and 7. There was a minimal loss of spermatogonia on PND 16 and 21 and mild degeneration of the | Barlow and Foster, 2001 |

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| seminiferous epithelium on PND 45, which had progressed to severe degeneration by PND 70. Taken together, these findings suggest that DBP disrupts androgen signaling leading to altered "imprinting" of androgen-dependent tissues and lack of development of reproductive organs manifested later in postnatal life. Moreover, the severity of the testicular lesions is not as marked in the early postnatal period, i.e., before the testes are fully functional. | |
| **Type/Objective:** Previously, we reported that DBP and MBuP induced cytotoxicity and inhibition of cell differentiation in cultured rat embryonic limb bud cells in a dose-dependent manner. In the present study, we analyzed the cell cycle and examined the effects of changes in cell cycle regulators on DBP-induced cytotoxicity and inhibition of differentiation in limb bud cells.<br>**Conclusion:** These results demonstrate that DBP or MBuP induces cytotoxicity and inhibition of differentiation in rat embryonic limb bud cells by accumulating cells in the G1 phase and inducing apoptosis.<br>**Method:** The micromass cell culture method for rat embryonic cells, developed by Flint, has been extensively used as an in vitro test for developmental toxicants.<br>**Results:** Both DBP and MbuP caused slight accumulation of cells in the G1 phase of the cell cycle and increased the sub-G1 population after 1, 2, and 4 days of culture in rat embryonic limb bud cells. DBP and MBuP downregulated the expression of the cyclin D1 protein but did not affect the expression of Cdk4. Exposure of limb bud cells to DBP and MBuP also induced apoptotic cell death in a terminal deoxynucleotidyl transferase (TdT)-mediated biotin-dUTP-digoxigenin nick-end labeling (TUNEL) assay. We also observed a decrease in the expression level of the poly (ADP ribose) polymerase (PARP) proform in western blot analysis. | Choi et al, 2002 |
| **Type/Objective:** Study on antiandrogenic effects of DBP and MBP<br>**Conclusion:** Findings suggest that MBP may be responsible for the developmental effects of DBP.<br>**Method:** Pregnant Wistar rats were dosed orally on GD 7-15 with 250, 500, 750, 1000, and 1250 mg/kg of DBP or MBP.<br>**Results:** The spectrum of fetal malformations, dependence on gestational days of treatment on the manifestation of teratogenicity, and decreased AGD and increased incidence of fetuses with undescended testes in male fetuses observed after the administration of DBP were in good agreement with those observed after the administration of MBP. These findings suggest that MBP may be responsible for the developmental effects of DBP. The doses that produced a decrease in the AGD and undescended testes in male offspring were lower than those producing maternal toxicity, fetal malformations, and postimplantation loss. The male reproductive system may be more susceptible than other organ systems to DBP toxicity after maternal exposure. | Ema, 2002 |
| **Type/Objective:** To examine whether testicular toxicity in rats is caused by a direct effect of MBP or by a secondary effect attributed to a hypoxic condition due to the MBP-induced hemoglobin deprivation<br>**Conclusion:** Results support the idea that the toxicity might be caused by hypoxia and a coincident depletion of SUDH activity, followed by an apoptotic testicular cell death.<br>**Method:**, The testes were perfused with a solution of MBP in Eagle's MEM or the MEM with/without oxygen, and the activities of testicular enzymes were measured.<br>**Results:** A decrease in the succinate dehydrogenase (SUDH) activity was observed by the hypoxic perfusate (20-30% dissolved oxygen (DO)), and an | Watanabe et al, 2002 |

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| induction of apoptosis was observed by the 7% DO perfusate. However, the 100 mM MBP perfusate decreased the activity of SUDH per testis weight, but not per protein level. | |
| **Type/Objective:** In the fetus, the effects of DBP (an antiandrogen) are mediated, not by interaction with the androgen receptor, but rather through diminution of testosterone (T) production by the testes. Previous studies have shown that several genes involved in cholesterol transport and steroidogenesis are downregulated at the mRNA level following in utero exposure to DBP. The purpose of this study was to make a functional determination of the points in the cholesterol transport and steroidogenesis pathways affected by DBP.<br>**Conclusion:** These data indicate that the toxic effects of DBP on the fetal testis are mediated at the level of cholesterol cleavage by P450 scc and possibly at the level of cholesterol transport into the mitochondria.<br>**Method:** We cultured fetal testis explants with T precursors and assessed cholesterol uptake and T production. Pregnant Sprague-Dawley rats were treated with 500 mg/kg DBP or corn oil control via oral gavage from gestational days 12 to 19. Following the final treatment, testes were removed from the fetuses and cultured for 3 h with 3H-cholesterol, leuteinizing hormone (LH), Bt2-cAMP, 22(R)-hydroxycholesterol, pregnenolone, progesterone, or 17-hydroxyprogesterone.<br>**Results:** T production in unsupplemented cultures of DBP-exposed testis was roughly 10% of that seen in corn oil controls (164.7 +/- 32 pg/h vs. 1684.1 +/- 347 pg/h). Both control and treated explants could be stimulated by LH or Bt2-cAMP, but T production by DBP-treated testes remained less than 50% of control levels. Incorporation of 3H-cholesterol by mitochondria of DBP-treated explants was 67% of that observed in controls, although this difference was not statistically significant ($p = 0.08$). Pregnenolone, progesterone, and 17-hydroxyprogesterone all significantly increased T production compared to unsupplemented DBP-treated explants. However, there was no significant difference between the unsupplemented explants and those treated with the membrane-permeable 22(R)-hydroxycholesterol. | Thompson et al, 2003 |
| **Type/Objective:** We evaluated sequelae in male rabbits following exposure to DBP at a dose known to adversely affect testicular function in rodents without causing systemic toxicity. Rabbits were used because they have a relatively long phase of reproductive development simulating better than rodents the reproductive development of humans, and because their use facilitates multiple evaluations of mating ability and seminal quality.<br>**Conclusion:** DBP induces lesions in the reproductive system of the rabbit, with the intrauterine period being the most sensitive stage of life.<br>**Method:** Rabbits were exposed to 0 or 400 mg DBP/kg/day on GD 15-29 or during PND 4-12, and male offspring were examined at 6, 12, and 25 weeks of age. Another group was exposed after puberty (for 12 weeks) and examined at the conclusion of exposure.<br>**Results:** The most pronounced reproductive effects were in male rabbits exposed in utero. Male offspring in this group exhibited reduction in numbers of ejaculated sperm (down 43%; $p < 0.01$), in weights of testes (at 12 weeks, down 23%; $p < 0.05$) and in accessory sex glands (at 12 and 25 weeks, down 36%; $p < 0.01$ and down 27%; $p < 0.05$, respectively). Serum testosterone levels were down (at 6 weeks, 32%; $p < 0.05$); a slight increase in histological alterations of the testis ($p < 0.05$) and a doubling in the percentage (from 16 to 30%, $p < 0.01$) of abnormal sperm; and 1/17 males manifesting hypospadias, hypoplastic prostate, and | Higuchi et al, 2003 |

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| cryptorchid testes with carcinoma in situ-like cells. In the DBP group exposed during adolescence, basal serum testosterone levels were reduced at 6 weeks ($p < 0.01$) while at 12 weeks, testosterone production in vivo failed to respond normally to a GnRH challenge ($p < 0.01$). In addition, weight of accessory sex glands was reduced at 12 weeks but not at 25 weeks after a recovery period; there was a slight increase in the percentage of abnormal sperm in the ejaculate; and 1/11 males was unilaterally cryptorchid. In both of these DBP-treated groups, daily sperm production, epididymal sperm counts, mating ability, and weights of body and nonreproductive organs were unaffected. | |
| **Type/Objective:** In utero exposure to 500 mg/kg/day DBP on GD 12-21 inhibits androgen biosynthesis, resulting in decreased fetal testicular testosterone (T) levels. One consequence of prenatal DBP exposure is malformed epididymides (~50%) in adult rats. Reduced fetal T levels may be responsible for the malformation since T is required for Wolffian duct stabilization and their development into epididymides. The objective of this study was to identify changes in gene expression associated with altered morphology of the proximal Wolffian duct following in utero exposure to DBP.<br>**Conclusion:** Results are suggestive of altered paracrine interactions between ductal epithelial cells and the surrounding mesenchyme during Wolffian duct differentiation due to lowered T production.<br>**Method:** Pregnant Crl:CD(SD)BR rats were gavaged with corn oil vehicle or 500 mg/kg/day DBP from GD 12 to GD 19 or 21.<br>**Results:** On GD 21, 89% of male fetuses in the DBP dose group showed marked underdevelopment of Wolffian ducts characterized by decreased coiling. RNA was isolated from Wolffian ducts on GD 19 and 21 and gene expression was examined using cDNA microarrays. These analyses identified several gene pathways involved in tissue differentiation that may be associated with the morphological changes observed on GD 21. Changes in mRNA expression within the insulinlike growth factor (IGF) pathway, matrix metalloproteinase (MMP) family, components of the extracellular matrix, and other developmentally conserved signaling pathways were also analyzed by real-time RT-PCR. On GD 19, immunolocalization of IGF-1 receptor protein demonstrated an increase in cytoplasmic expression in the mesenchymal and epithelial cells. There was also a variable decrease in androgen receptor protein in ductal epithelial cells on GD 19. This study provides valuable insight into the effects of antiandrogens on the molecular mechanisms involved in Wolffian duct development. | Bowman et al, 2004 |
| **Type/Objective:** Exposure to DBP in utero impairs the development of the male rat reproductive tract. The adverse effects are due in part to a coordinated decrease in expression of genes involved in cholesterol transport and steroidogenesis with a resultant reduction in testosterone production in the fetal testis. The objective here was to determine the dose-response relationship for the effect of DBP on steroidogenesis in fetal rat testes.<br>**Conclusion:** Our results demonstrate a coordinate, dose-dependent reduction in the expression of key genes and proteins involved in cholesterol transport and steroidogenesis and a corresponding reduction in testosterone in fetal testes following maternal exposure to DBP, at dose levels below which adverse effects are detected in the developing male reproductive tract.<br>**Method:**, Pregnant Sprague-Dawley rats received corn oil (vehicle control) or DBP (0.1, 1.0, 10, 50, 100, or 500 mg/kg/day) by gavage daily from gestation day (GD) 12 to 19. Testes were isolated on GD 19, and changes in gene and protein expression were quantified by RT-PCR and Western analysis. Fetal testicular | Lehmann et al, 2004 |

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| testosterone concentration was determined by radioimmunoassay. DBP exposure resulted in significant dose-dependent reductions in mRNA and protein concentration of scavenger receptor, steroidogenic acute regulatory protein (StAR), cytochrome P450 side-chain cleavage, 3beta-hydroxysteroid dehydrogenase, and cytochrome P450c17.<br>**Results:** Testicular testosterone was reduced at doses of 50 mg/kg/day and above. Whole-testis expression of peripheral benzodiazepine receptor (PBR) mRNA, which functions with StAR to transport cholesterol across the mitochondrial membrane, was upregulated following exposure to DBP at 500 mg/kg/day. By immunocytochemistry, however, PBR protein was reduced in interstitial cells and also expressed but not reduced in gonocytes. | |
| **Type/Objective:** Study of effects of DBP on fetal liver<br>**Conclusion:** The results indicate that hepatic steroid- and xenobiotic-metabolizing enzymes are susceptible to DBP induction at the fetal stage; such effects on enzyme expression are likely mediated by xenobiotic-responsive transcriptional factors, including CAR and PXR. DBP is broadly reactive with multiple pathways involved in maintaining steroid and lipid homeostasis.<br>**Method:** Pregnant Sprague-Dawley rats were orally dosed with DBP at levels of 10, 50, or 500 mg/kg/day from GD 12 to 19; maternal and fetal liver samples were collected on GD 19 for analyses.<br>**Results:** Increased protein and mRNA levels of CYP 2B1, CYP 3A1, and CYP 4A1 were found in both maternal and fetal liver at 500mg/kg/day. DBP at high doses also caused an increase in the mRNA of hepatic estrogen sulfotransferase and UDP-glucuronosyltransferase 2B1 in the dams but not in the fetuses. Xenobiotic induction of CYP3A1 and 2B1 is known to be mediated by the nuclear hormone receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR). In vitro transcriptional activation assays showed that DBP activates both PXR and CAR. The main DBP metabolite, mono-**butyl-phthalate** (MBP) did not interact strongly with either CAR or PXR. | Wyde et al, 2005 |
| **Type/Objective:** To identify signalling pathways associated with DBP-induced testicular dysgenesis and to determine the region-specificity of the gene expression alterations.<br>**Conclusion:** These results indicate that DBP-induced testicular dysgenesis involves region- and cell- type-specific effects on a number of genes many of which are regulated by nuclear hormone receptors.<br>**Method:** Transcriptional profiling of RNA isolated from laser capture microdissected interstitial (INT) and tubular (TUB) regions of foetal testes of Wistar rats exposed in utero to 500 mg DBP/kg was performed. High density microarray analysis (rat whole genome array) in foetal testes at gestational day (GD) 19 of RNA isolated from INT orTUB regions identified genes that were significantly regulated (signature lists, $P<0.01$). Luminator software was used to compare signature gene lists.<br>**Results:** This analysis identified genes that were uniquely or commonly regulated by DBP in the two regions. Effects on pathways regulating steroidogenesis (StAR, INHA), cholesterol synthesis (HMGCS, IDI), fatty acid oxidation (SCD) and testes morphogenesis (CRABP2, FAT) were focussed to the INT (Leydig cell) region. By contrast genes involved in Mullerian duct regression (AMH), chromatin bending (HMGB1, HMGN2), phagocytosis (MARKS) and the response to hypoxia (HIF1A) were uniquely altered in the TUB (Sertoli cell) region. Genes that were identified as being commonly regulated by DBP in both testicular regions were associated with steroidogenesis (DBI, FABP5, SCARB1, | Plummer et al, 2006 |

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| Cyp 17A) and cell/tissue assembly (PHGDH, ARPC5, SERPING1). Immunohistochemical analysis of foetal testes confirmed the region-specificity of RNA-level alterations to several genes at the protein level. For example, CRAPB2 and PEBP, were specifically down-regulated in Leydig cells. | |
| **Type/Objective:** The aims of present study were to compare the effects of in utero exposure of several chemicals which have antiandrogenic characteristics on the development of reproductive organs and to investigate the specific mechanisms related to the abnormalities observed in the male reproductive system.<br>**Conclusion:** These results demonstrate that exposure to antiandrogen during gestation days 10-19 causes changes in the endocrine system resulting in abnormal development of male reproductive organs.<br>**Method:** During gestation days 10-19, pregnant Sprague-Dawley (SD) female rats were administered orally with corn oil (control), or flutamide (1, 12.5, or 25 mg/kg/day) Di(n-butyl)phthalate (DBP) (250, 500, or 700 mg/kg/day).<br>**Results:** At 31 of age, the SD male rats reproductive tract abnormalities (hypospadias, cryptorchidism) were dose-dependently increased in the DBP or flutamide treated groups. Preputial separation (PPS) was delayed by DBP (250, 500, or 700 mg/kg/day) or flutamide (1, 12.5, or 25 mg/kg/day) treatment. In ventral prostate epithelium cells, expression of androgen receptor and NKx3.1 were decreased in the DBP (700 mg/kg/day) or flutamide (25 mg/kg/day) treated groups. In cDNA microarray analysis, expression of hydroxysteroid dehydrogenase 17beta and kruppel-like factor were decreased in ventral prostate treated on the DBP (700 mg/kg/day) or flutamide (25 mg/kg/day). At 31 days of age, the serum IGF-I, estradiol, and dihydrotestosterone (DHT) levels significantly decreased in the DBP (700 mg/kg/day) and flutamide (25 mg/kg/day) treated groups. In the expression of AR and Shh in the penis were decreased on the DBP (700 mg/kg/day) or flutamide (25 mg/kg/day) treated groups. In cDNA microarray analysis, IGF-II, homeobox 2, EGF gene expression were dose-dependently decreased on the DBP (700 mg/kg/day) or flutamide (25 mg/kg/day) treated groups. In addition, DBP and flutamide treated groups dose dependently decreased the expression of IGF-I and IGF-II in the undescended testis respectively. | Kang et al, 2006 |
| **Type/Objective:** DBP and DEHP have similar modes of action: in utero exposure reduces testosterone (T) production in fetal male rats, inhibits reproductive tract differentiation, and induces reproductive organ malformations. In utero exposure to DBP or DEHP also decreases expression of insulin-like factor 3 (insl3), a hormone responsible for gubernacular ligament development. We hypothesized that (1) co-administered DBP and DEHP would act in a cumulative fashion to induce reproductive malformations, and (2) cumulative changes in fetal steroid hormones and expression of genes responsible for insl3 and steroid production would enhance the incidence of reproductive malformations in adulthood.<br>**Conclusion:** These data indicate that individual anti-androgenic phthalates with a similar mode of action can elicit cumulative effects on fetal testis hormone production and reproductive tract differentiation when administered as a mixture.<br>**Method:** Pregnant rats were gavaged on gestation days (GD) 14-18 with vehicle control, 500 mg/kg DBP and/or DEHP. In experiment one, adult male offspring were necropsied, and reproductive malformations and androgen-dependent organ weights were recorded. In experiment two, GD18 fetal testes were incubated for T production, and processed for gene expression by qrtPCR.<br>**Results:** The DBP+DEHP dose increased the incidence of reproductive | Howdeshell et al, 2006 |

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| malformations in a cumulative fashion with the chemicals acting in an additive manner. Androgen-dependent organ weights also exhibited decreases in the DBP+DEHP combination dose. Fetal T, and the expression of insl3 and genes in the steroidogenic pathway (steroidogenic acute regulatory protein and cyp11a) were significantly reduced by DEHP and further decreased by the DBP+DEHP dose. | |
| **Type/Objective:** To investigate the gene expression profiles in testes of male rats given DBP orally for 30 days<br>**Conclusion:** DBP can significantly affect the testicular gene expression profiles involved in steroidogenesis and spermatogenesis affecting testicular growth and morphogenesis.<br>**Method and Results:** Sprague Dawley male rats were orally given 250, 500, or 750 mg DBP/kg/day for 30 days. Testes weights in the 500 and 750 mg/kg/day rats significantly reduced. Using GeneFishing PCR on total RNA that was isolated from these males, 56 differentially expressed genes were seen in the 750 mg/kg/day dosed rat testes. The known genes were involved in xenobiotic metabolism, testis development, sperm maturation, steroidogenesis, and immune response, as well as the up regulation of peroxisome proliferation and lipid homeostasis genes. Using RTPCR, they found that the LDHA and Spag4 genes were significantly increased, and the PBR gene was significantly decreased in a dose dependent manner. They also found that at the highest dose, 750 mg/kg/day, steroidogenic related genes SR-B1, StAR, P450scc and Cyp17 were significantly increased, while CYP19 was significantly decreased at 250 and 750 mg/kg/day DBP. Because these genes may play a significant role in cholesterol transport and steroidogenic pathways, testosterone levels were examined by RIA. Serum testosterone levels showed a decrease in all DBP treatment groups, but none were statistically significant. Ryu et al. also evaluated the expression of TR-α1, AR and ERβ proteins using western blot analysis and RTPCR. They found that the expression of TR-α1 was dose dependently increased, while AR and ERβ were significantly decreased in the 500 and 750 mg/kg/day exposure groups. In addition, protein expression of PPARγ was significantly increased at the highest dose, while RXR-γ remained unchanged. All reported statistically significant findings were $p<0.05$. | Ryu et al, 2007, as summarized in CPSC, 2010 |
| **Type/Objective:** The goal of this study was to elucidate mechanisms of phthalate toxicity in normal human cells to provide information concerning interindividual variation and gene-environment interactions.<br>**Conclusion:** Data from this study will help clarify the role of DBP in reproductive toxicity, and yield biomarkers of exposure for future epidemiology studies.<br>**Method:** Normal human mammary epithelial cell strains were obtained from discarded tissues following reduction mammoplasty [Cooperative Human Tissue Network (sponsors: NCI/NDRI)]. Gene transcription in each cell strain was analyzed using high-density oligonucleotide DNA microarrays (U133A, Affymetrix) and changes in the expression of selected genes were verified by real-time polymerase chain reaction (PCR) (ABI). DNA microarrays were hybridized with total RNA that was collected after DBP treatment for 5 hr and 10 hr. RNA was harvested from the vehicle control (acetone) at 10 hr. Data Mining Tool software (Affymetrix) was used to separate genes in clusters based on their expression patterns over time.<br>**Results:** Only 57 genes were found to be altered in all four cell strains following exposure to DBP. These included genes involved in fertility (inhibin, placental | Gwinn et al, 2007 |

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| growth factor), immune response (tumor necrosis factor induced protein), and antioxidant status (glutathione peroxidase). | |
| **Type/Objective:** The time-response effects of di(n-butyl) phthalate (DBP) on the expression patterns of the testicular genes in male Sprague-Dawley rats were examined for different periods of exposure (1, 7, 14, or 28 d).<br>**Conclusion:** These results suggest that the acute and chronic effects of DBP on the steroidogenic pathways in the testes show mechanistically distinct patterns. Data thus provide some insights into the molecular mechanisms underlying DBP-induced testicular dysgenesis.<br>**Method:** The steroidogenic- or spermatogenic-related gene expression patterns were measured using reverse-transcription polymerase chain reaction (RT-PCR).<br>**Results:** After 28 d of exposure, the serum concentrations of DBP and monobutyl phthalate (MBP) increased in a dose-dependent manner, and were significantly higher in the DBP-treated rats than in the control rats. Liver weight was increased markedly at 28 d after DBP exposure at 750 mg/kg/d. Testicular weight was reduced significantly after 14 and 28 d of exposure. DBP (750 mg/kg/d) produced a significant increase in scavenger receptor class B1 (SR-B1) and steroidogenic acute regulatory (StAR) mRNA after 14 and 28 d of exposure. The level of cytochrome P-450 (P450) side-chain cleavage (P450scc) mRNA decreased in the group treated with DBP at 750 mg/kg/d at 7 d. After 14 and 28 d of exposure, there was an apparent increase in P450scc mRNA. High doses of DBP significantly increased the Cyp17 mRNA level after 28 d of exposure. At 7 d, a significant decrease in Cyp19 mRNA was observed only in the group exposed to 750 mg/kg/d DBP. In addition, DBP significantly decreased the levels of a spermatid-specific gene (Spag4) and lactate dehydrogenase A (LDHA) mRNA after 7 d of exposure. The levels of androgen receptor (AR), estrogen receptor-alpha (ER-alpha), and retinoid X receptor-gamma (RXR-r) expression decreased significantly in a time- or dose-dependent manner. DBP significantly increased the peroxisome proliferator-activated receptor-gamma (PPAR-r) and phosphorylated extracellular-signal-regulated kinase (p-ERK1/2) levels in the testis. | Ryu et al, 2008 |
| **Type/Objective:** The objective of this study was to evaluate the developmental abnormalities and carry out the molecular analysis of external genitalia in newborn hypospadiac male rats induced by maternal exposure to DBP.<br>**Conclusion:** The reproductive system and development conditions of newborn hypospadiac rats were damaged by DBP. These disturbed signaling pathways which orchestrating genital development might play an important role in the toxic process of DBP induced hypospadias.<br>**Method:** Timed-pregnant rats were given DBP by gastric intubation at dose of 750 mg/kg body weight (bw)/day from GD 14-18 to establish a hypospadiac rat model.<br>**Results:** The incidence of hypospadias was 46.67% in male offsprings. On postnatal day (PND) 7, at the newborn stage, decreased body weight and anogenital distance (AGD)/body weight ratio were observed in newborn hypospadiac male rats. The general image and transverse serial histological analysis of genitalia of newborn hypospadiac male rats confirmed the malformation. Autopsy analysis revealed development of reproductive organs (testes, genital tubercle (GT)), hollow organs (stomach, bladder), and solid organs (brain, heart, liver, spleen, lung, kidney, pancreas) in newborn hypospadiac male rats affected by DBP. Moreover, significantly decreased gene expression of important signaling molecules necessary for GT formation including sonic | Zhu et al, 2009 |

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| hedgehog signaling molecules (Shh and Ptched 1), bone morphogenetic proteins signaling molecules (Bmp4 and Bmp7), fibroblast growth factor signaling molecules (Fgf8, Fgf10 and Fgfr2), and the transforming growth factor-beta superfamily signaling molecules (TGF-beta1 and TGF-beta receptor III) were observed, for the first time, in the GT of newborn hypospadias induced by DBP. | |
| **Type/Objective:** Mammalian receptors and assay systems are generally used for in vitro screening of endocrine-disrupting chemicals with the assumption that minor differences in amino acid sequences among species do not translate into significant differences in receptor function. Objectives of the present study were to evaluate the performance of two different in vitro assay systems (a whole cell and a cell-free competitive binding assay) in assessing whether binding of chemicals differs significantly between full-length recombinant estrogen receptors from fathead minnows (fhERalpha) and those from humans (hERalpha).<br>**Conclusion:** No strong evidence showed species-specific binding among the chemicals tested.<br>**Method: and Results:** It was confirmed that 17beta-estradiol displays a reduction in binding to fhERalpha at an elevated temperature (37 degrees C), as has been reported with other piscine estrogen receptors. Several of the chemicals (17beta-estradiol, ethinylestradiol, alpha-zearalanol, fulvestrant, dibutyl phthalate, benzyl butyl phthalate, and cadmium chloride) displayed higher affinity for fhERalpha than for hERalpha in the whole cell assay, while only dibutyl phthalate had a higher affinity for fhERalpha than for hERalpha in the cell-free assay. Both assays were effective in identifying strong binders, weak binders, and nonbinders to the two receptors. | Rider et al, 2009 |
| **Type/Objective:** The purpose of this study was to determine the effects of DBP administration on male reproductive organ development in F1 Sprague-Dawley rats following in utero exposure.<br>**Conclusion:** Data demonstrated that in utero exposure to DBP produced several abnormal responses in male reproductive organs, and these effects may be due to disruption of the stage-specific expression of genes related to androgen-dependent organs development.<br>**Method:** During gestation days (GD) 10-19, pregnant rats were administered daily, orally, DBP at 250, 500, or 700 mg/kg or flutamide (1, 12.5, or 25 mg/kg/d) as a positive control. The male offspring were sacrificed at 31 d of age. DBP and flutamide dose-dependently significantly increased the incidence of hypospadias and cryptorchidism in F1 male offspring.<br>**Results:** The weights of testes and accessory sex organs (epididymides, seminal vesicles, ventral prostate, levator ani plus bulbocavernosus muscles (LABC), and Cowper's glands) were significantly reduced in DBP-treated animals. Furthermore, cauda agenesis of epididymides and ventral prostate atrophy were observed in high-dose 700-mg/kg DBP males. Anogenital distance (AGD) and levels of dihydrotestosterone (DHT) and testosterone were significantly decreased in the DBP (700 mg/kg/d)-treated groups. In particular, the expression of androgen receptor (AR) and 5α-reductase type 2 in the proximal penis was markedly depressed following administration of DBP (700 mg/kg/d) or flutamide (25 mg/kg/d). The expression of sonic hedgehog (Shh) in the urethral epithelium of the proximal penis was significantly less in the DBP (700 mg/kg/d)- or flutamide (25 mg/kg/d)-treated groups. In addition, DBP dose-dependently significantly increased the expression of estrogen receptor (ER α) in the undescended testis. | Kim et al, 2010 |
| **Type/Objective:** The present study was designed to further investigate the | Zhou et al, 2010 |

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| potential male reproductive toxicity of DBP . Oxidative stress was assessed in rat testes as an underlying mechanism.<br>**Conclusion:** DBP alters the testicular structure and function, at least partly, by inducing oxidative stress in testes of adult rats.<br>**Method:** Forty SD adult rats were randomly allotted to four groups, and DBP was administered to each group by oral gavage at doses of 0 (control), 100, 250, and 500 mg/kg/d for 2 consecutive weeks.<br>**Results:** The results indicated that the reproductive toxicity of DBP is dose-dependent. Body and testicular weight was significantly decreased in rats of DBP exposure at a dose of 500 mg/kg/d. Sperm count and motility were significantly decreased at doses of 250 and 500 mg/kg/d. The same two doses significantly inhibited the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione (GSH) while the level of malondialdehyde (MDA) was significantly increased in testes of rats. Microscopy with hematoxylin and eosin (HE) staining showed that seminiferous tubules atrophy and seminiferous epithelial cells disintegrated and shed in rats of DBP exposure at doses of 500 mg/kg/d. | |
| **Type/Objective:** To investigate the effect of DBP on structure and function of epididymis in adult male rats by histological and biochemical study. Oxidative stress was also assessed in rat epididymis as an underlying mechanism.<br>**Conclusion:** DBP exposure alters the epididymal structure and function by inducing oxidative stress in epididymis of adult rats.<br>**Method:** Forty SD adult rats were randomly allotted to four groups, and DBP was administered to each group by oral gavage at doses of 0 (control), 100, 250 and 500 mg/kg/day for 2 consecutive weeks.<br>**Results:** The results indicated that the epididymal toxicity of DBP is dose-dependent. Epididymal weight, activities of epididymal alpha-glucosidase and glutathione peroxidase (GSH-Px) was significantly decreased in rats of 500 mg/kg DBP exposure group compared to the control. The activity of superoxide dismutase (SOD) was significantly decreased while the level of malondialdehyde (MDA) was significantly increased in the epididymal tissue of the 250 and 500 mg/kg DBP exposure groups compared with the control group. Moreover, microscopy with hematoxylin and eosin (HE) staining showed that atrophy of epididymal tubules, the interstitial vascular was hyperemia and the lumina were oligozoospermic in rats of 500 mg/kg DBP exposure group. | Zhou et al, 2011 |
| **Type/Objective:** Human testicular germ-cell cancer (TGCC) has foetal origins and may be one component of a testicular dysgenesis syndrome (TDS). Certain phthalates induce TDS in rats, including effects on foetal germ cells (GC). As humans are widely exposed to phthalates, study of the effects of phthalates on foetal rat GC could provide an insight into the vulnerability of foetal GC to disruption by environmental factors, and thus to origins of TGCC. This study has therefore characterized foetal GC development in rats after in utero exposure to DBP.<br>**Conclusion:** DBP differentially affects foetal GC in rats according to stage of gestation, effects that may be relevant to the human because of their nature (OCT4, DMRT1 effects) or because similar effects are demonstrable in vitro on human foetal testes (GC number).<br>**Method:** GC number, proliferation, apoptosis, differentiation (loss of OCT4, DMRT1 expression, DMRT1 re-expression, GC migration) and aggregation were evaluated at various foetal and postnatal ages.<br>**Results:** DBP exposure reduced foetal GC number by ~60% by e15.5 and | Jobling et al, 2011 |

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| prolonged GC proliferation, OCT4 and DMRT1 immunoexpression; these effects were induced in the period immediately after testis differentiation (e13.5-e15.5). In contrast, DBP-induced GC aggregation stemmed from late gestation effects (beyond e19.5). Foetal DBP exposure delayed postnatal resumption of GC proliferation, leading to bigger deficits in numbers, and delayed re-expression of DMRT1 and radial GC migration. | |
| **Type/Objective:** To first explore the roles of Wnt/β-catenin pathway in the fetal rat genital tubercle (GT) following in-utero exposure to DBP.<br>**Conclusion:** These findings, for the first time, indicate that DBP may affect the development of GT by down-regulating the Wnt/β-catenin pathway in fetal male rats.<br>**Method:** Timed-pregnant rats were given DBP by gastric intubation at a dose of 750 mg/kg body weight (bw)/day from gestation day (GD) 14 to GD18 to establish a rat model of hypospadias.<br>**Results:** On GD19, genital tubercle down-regulation of β-catenin, Phospho-GSK-3β, and up-regulation of GSK-3β (glycogen synthase kinase-3β), NFκB in fetal male rats was observed by western blot analysis. β-catenin was located in the urethral plate epithelium (UPE). Immunochemistry showed that the relative expression of β-catenin decreased in the DBP-treated fetal rat GT compared to the normal control. | Zhang et al, 2011 |
| **Type/Objective:** Fetal rat phthalate exposure produces a spectrum of male reproductive tract malformations downstream of reduced Leydig cell testosterone production, but the molecular mechanism of phthalate perturbation of Leydig cell function is not well understood.<br>**Conclusion:** Together, these data suggest that phthalate-induced inhibition of fetal testis steroidogenesis is closely associated with reduced activity of several lipid metabolism pathways and SREBP2-dependent cholesterologenesis in Leydig cells.<br>**Method and Results:** By bioinformatically examining fetal testis expression microarray data sets from susceptible (rat) and resistant (mouse) species after DBP exposure, we identified decreased expression of several metabolic pathways in both species. However, lipid metabolism pathways transcriptionally regulated by sterol regulatory element-binding protein (SREBP) were inhibited in the rat but induced in the mouse, and this differential species response corresponded with repression of the steroidogenic pathway. In rats exposed to 100 or 500 mg/kg DBP from gestational days (GD) 16 to 20, a correlation was observed between GD20 testis steroidogenic inhibition and reductions of testis cholesterol synthesis endpoints including testis total cholesterol levels, Srebf2 gene expression, and cholesterol synthesis pathway gene expression. SREBP2 expression was detected in all fetal rat testis cells but was highest in Leydig cells. Quantification of SREBP2 immunostaining showed that 500 mg/kg DBP exposure significantly reduced SREBP2 expression in rat fetal Leydig cells but not in seminiferous cords. By Western analysis, total rat testis SREBP2 levels were not altered by DBP exposure. | Johnson et al, 2011 |
| **Type/Objective:** Fibroblast growth factor 8 (FGF8) is an androgen-induced growth factor (AIGF) that is crucial for embryonic development. This study was developed to investigate the role of FGF8 in developmental abnormalities of the genital tubercle (GT) in hypospadiac male rats when prenatally exposed to DBP.<br>**Conclusion:** Collectively, these data clearly demonstrate an interaction between androgen and FGF8, which might play an important role in the occurrence of hypospadias and abnormal organ development induced by DBP. | Liu et al, 2012 |

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| **Method:** DBP was administered to timed-pregnant rats to establish the hypospadiac rat model where the incidence of hypospadias in male offspring was 43.6%.<br>**Results:** On postnatal day (PND) 7, decreased mRNA and protein expression levels for androgen receptor (AR) and FGF8 were observed in the GT of hypospadiac rats. Decreased serum testosterone (T) levels were observed in groups displaying hypospadias, which was confirmed using histological analysis. Further anatomical examination using digital photography helped to reveal visualized expression of dysplasia in organs strongly associated with hypospadias. In addition, changes in body weight (BW) and anogenital distance (AGD) were recorded, showing definitive decreases. | |
| **Type/Objective:** Reproductive disorders that are common/increasing in prevalence in human males may arise because of deficient androgen production/action during a fetal 'masculinization programming window'. We identify a potentially important role for Chicken Ovalbumin Upstream Promoter-Transcription Factor II (COUP-TFII) in Leydig cell (LC) steroidogenesis that may partly explain this.<br>**Conclusion:** These findings suggest that lifting of repression by COUP-TFII may be an important mechanism that promotes increased testosterone production by fetal LC to drive masculinization. As we also show an age-related decline in expression of COUP-TFII in human fetal LC, this mechanism may also be functional in humans, and its susceptibility to disruption by environmental chemicals, stress and pregnancy hormones could explain the origin of some human male reproductive disorders.<br>**Method and Results:** In rats, fetal LC size and intratesticular testosterone (ITT) increased ~3-fold between e15.5-e21.5 which associated with a progressive decrease in the percentage of LC expressing COUP-TFII. Exposure of fetuses to DBP, which induces masculinization disorders, dose-dependently prevented the age-related decrease in LC COUP-TFII expression and the normal increases in LC size and ITT. We show that nuclear COUP-TFII expression in fetal rat LC relates inversely to LC expression of steroidogenic factor-1 (SF-1)-dependent genes (StAR, Cyp11a1, Cyp17a1) with overlapping binding sites for SF-1 and COUP-TFII in their promoter regions, but does not affect an SF-1 dependent LC gene (3β-HSD) without overlapping sites. We also show that once COUP-TFII expression in LC has switched off, it is re-induced by DBP exposure, coincident with suppression of ITT. Furthermore, other treatments that reduce fetal ITT in rats (dexamethasone, diethylstilbestrol (DES)) also maintain/induce LC nuclear expression of COUP-TFII. In contrast to rats, in mice DBP neither causes persistence of fetal LC COUP-TFII nor reduces ITT, whereas DES-exposure of mice maintains COUP-TFII expression in fetal LC and decreases ITT, as in rats. | van den Driesche et al, 2012 |
| **Type/Objective:** A case study was conducted, using DBP, to explore an approach to using toxicogenomic data in risk assessment. The toxicity and toxicogenomic data sets relative to DBP-related male reproductive developmental outcomes were considered conjointly to derive information about mode and mechanism of action.<br>**Conclusion:** This case study serves as an example of the steps that can be taken to develop a toxicological data source for a risk assessment, both in general and especially for risk assessments that include toxicogenomic data.<br>**Method:** We describe the case study evaluation of the toxicological database for DBP, focusing on identifying the full spectrum of male reproductive developmental effects. The data were assessed to 1) evaluate low dose and low incidence findings and 2) identify male reproductive toxicity endpoints without | Makris et al, 2013 |

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| well-established modes of action (MOAs).<br>**Results:** These efforts led to the characterization of data gaps and research needs for the toxicity and toxicogenomic studies in a risk assessment context. Further, the identification of endpoints with unexplained MOAs in the toxicity data set was useful in the subsequent evaluation of the mechanistic information that the toxicogenomic data set evaluation could provide. The extensive analysis of the toxicology data set within the MOA context provided a resource of information for DBP in attempts to hypothesize MOAs (for endpoints without a well-established MOA) and to phenotypically anchor toxicogenomic and other mechanistic data both to toxicity endpoints and to available toxicogenomic data. | |
| **Type/Objective:** No studies have evaluated its effects on ovarian follicles. Therefore, we used a follicle culture system to evaluate the effects of DBP on antral follicle growth, cell cycle and apoptosis gene expression, cell cycle staging, atresia, and 17β-estradiol (E(2)) production.<br>**Conclusion:** These data suggest that DBP targets antral follicles and alters the expression of cell cycle and apoptosis factors, causes cell cycle arrest, decreases E(2), and triggers atresia, depending on dose.<br>**Method:** Antral follicles were isolated from adult CD-1 mice and exposed to DBP at 1, 10, 100, and 1000 μg/ml for 24 or 168 h.<br>**Results:** Follicles treated with vehicle or DBP at 1-100 μg/ml grew over time, but DBP at 1000 μg/ml significantly suppressed follicle growth. Regardless of effect on follicle growth, DBP-treated follicles had decreased mRNA for cyclins D2, E1, A2, and B1 and increased p21. Levels of the proapoptotic genes Bax, Bad, and Bok were not altered by DBP treatment, but DBP 1000 μg/ml increased levels of Bid and decreased levels of the antiapoptotic gene Bcl2. DBP-treated follicles contained significantly more cells in G(1) phase, significantly less cells in S, and exhibited a trend for fewer cells in G(2). Although DBP did not affect E(2) production and atresia at 24 h, follicles treated with DBP had reduced levels of E(2) at 96 h and underwent atresia at 168 h. | Craig et al, 2013 |
| **Type/Objective:** To investigate the neurotoxicity of perinatal exposure of DBP on rodent offspring.<br>**Conclusion:** These results may provide basic experimental evidence for better understanding the neurotoxic effects of DBP on neonatal, immature and mature brains.<br>**Method:** Pregnant rats received intragastric DBP (500 mg/kg/day) from GD 6 to PND 21. Animals in the control group received the same volume of edible corn oil. Brain sections or tissues from offspring rats on PND5, PND21 and PND60 were collected for analysis.<br>**Results:** Histological examination demonstrated that perinatal exposure of DBP resulted in hippocampal neuron loss and structural alternation in neonatal and immature offspring rats (PND5 and PND21), while no significant change was found in mature rats (PND60). DBP exposure induced cell apoptosis in hippocampal neurons of these neonatal and immature animals, as evidenced by the increased number of TUNEL-positive and Annexin V-propidium iodide (PI) positive cells and up-regulated caspase-3 activity. Moreover, DBP exposure decreased the expression of synaptophysin in the hippocampus and reduced both the slope and amplitude of field excitatory postsynaptic potentials (fEPSPs). DBP also impaired the spatial learning and memory of offspring rats. However, no significant difference in the susceptibility to DBP-induced neurotoxicity was found between male and female offspring rats. Our findings indicated that perinatal exposure of DBP could induce neurotoxicity in neonatal and immature | Li et al, 2013 |

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| offspring animals, but had no influence on mature animals after DBP withdrawal. | |
| **Type/Objective:** Study on neurotoxicity induced by perinatal exposure to DBP on the immature and mature offspring<br>**Conclusion:** Authors concluded that perinatal exposure of DBP could induce neurotoxicity in immature offspring rats through regulation of AROM, ER-β, BDNF and p-CREB expression, while it has no influence on mature offspring animals.<br>**Method:** Pregnant rats were given intragastric administration of 500mg/kg body weight DBP daily from gestational day 6 to postnatal day 21 while control animals received the same volume of edible corn oil. Serum estradiol and testosterone levels of the offspring were evaluated. Protein levels of AROM, ER-β, BDNF and p-CREB in the hippocampus were also measured.<br>**Results:** Perinatal exposure of DBP significantly up-regulated the serum estradiol levels in both immature and mature offspring rats. DBP exposure also significantly down-regulated the testosterone levels in immature male and female rats and mature male rats but had no influence on the testosterone levels in mature female rats. DBP exposure up-regulated AROM, but down-regulated ER-β, BDNF and p-CREB expression in the hippocampus of immature rat offspring, while it had no influence on the levels of these proteins in the mature animals. | Li et al, 2014 |
| **Type/Objective:** Recently, we reported that prenatal DBP exposure induced atypical Leydig cells (LCs) hyperplasia during adulthood. The present study investigated the expression of estrogen receptor α (ERα), estrogen receptor β (ERβ), and androgen receptor (AR) in LCs of rats exposed to DBP in utero.<br>**Conclusion:** Seminiferous tubule degeneration and atypical hyperplasia of LCs during adulthood in rats exposed in utero to DBP was associated with an increase in expression of ERα and a decrease of ERβ and AR in the testis.<br>**Method:** Recently, we reported that prenatal DBP exposure induced atypical Leydig cells (LCs) hyperplasia during adulthood. The present study investigated the expression of estrogen receptor α (ERα), estrogen receptor β (ERβ), and androgen receptor (AR) in LCs of 5-, 7-, 9-, 14-, and 17-week-old Sprague-Dawley (srl) rats whose dams had been administered DBP intragastrically at 100 mg/kg/day or the vehicle (corn oil) from days 12 to 21 postconception. Immunohistochemical,<br>**Results:** Western blotting, and reverse transcription polymerase chain reaction analyses revealed that the expressions of ERα, ERβ, and AR proteins and mRNAs in the DBP group were similar to those of the vehicle group at 5 and 7 weeks, but significantly higher ERα and lower ERβ and AR levels were observed in the DBP group at 9 to 17 weeks. The rats prenatally exposed to DBP had seminiferous tubule degeneration and atypical hyperplasia of LCs during adulthood, which was associated with an increase in expression of ERα and a decrease of ERβ and AR in the testis. | Wakui et al, 2014 |
| **Type/Objective:** This study identified gene expression changes following in utero DBP and flutamide exposures in Sprague-Dawley rat foreskin.<br>**Conclusion:** DBP induced changes in specific genes that were maintained after birth.<br>**Method:** Dams were exposed to 100 or 500mg/kg/day dibutyl phthalate or 5mg/kg/day flutamide from gestational days 16-20. Microarray analysis was performed on foreskin tissue from gestational day 20 and postnatal day 5.<br>**Results:** Expression changes found following DBP exposure were not present following flutamide treatment, indicating that expression changes were specific to DBP exposure and not caused by altered androgen signaling. Genes that were | Pike et al, 2014 |

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| expressed at lower levels in tissue from pups treated with the low dose of DBP were reduced more in pups treated with the high dose of DBP, demonstrating a dose response effect of this compound. Changes in expression of Marcks, Pum1, Nupr1, and Penk caused by in utero phthalate exposure were confirmed by qRT-PCR. Changes in expression of these genes were maintained after birth and consequently their expression could serve as markers of chemical exposure and biological response. | |
| **Type/Objective:** In utero exposure to antiandrogenic xenobiotics such as di-n-butyl phthalate (DBP) has been linked to congenital defects of the male reproductive tract, including cryptorchidism and hypospadias, as well as later life effects such as testicular cancer and decreased sperm counts. Experimental evidence indicates that DBP has in utero antiandrogenic effects in the rat. However, it is unclear whether DBP has similar effects on androgen biosynthesis in human fetal testis.<br>**Conclusion:** DBP did not affect androgenic endpoints in a human fetal testis xenograft.<br>**Method:** To address this issue, we developed a xenograft bioassay with multiple androgen-sensitive physiological endpoints, similar to the rodent Hershberger assay. Adult male athymic nude mice were castrated, and human fetal testis was xenografted into the renal subcapsular space. Hosts were treated with human chorionic gonadotropin for 4 weeks to stimulate testosterone production. During weeks 3 and 4, hosts were exposed to DBP or abiraterone acetate, a CYP17A1 inhibitor.<br>**Results:** Although abiraterone acetate (14 d, 75 mg/kg/d po) dramatically reduced testosterone and the weights of androgen-sensitive host organs, DBP (14 d, 500 mg/kg/d po) had no effect on androgenic endpoints. DBP did produce a near-significant trend toward increased multinucleated germ cells in the xenografts. Gene expression analysis showed that abiraterone decreased expression of genes related to transcription and cell differentiation while increasing expression of genes involved in epigenetic control of gene expression. DBP induced expression of oxidative stress response genes and altered expression of actin cytoskeleton genes. | Spade et al, 2014 |
| **Type/Objective:** We sought to identify the effects of phthalate exposure on human fetal germ cells in a dynamic model and to establish whether the rat is an appropriate model for investigating such effects.<br>**Conclusion:** Our findings provide the first comparison of DBP effects on germ cell number, differentiation, and aggregation in human testis xenografts and in vivo in rats. We observed comparable effects on germ cells in both species, but the effects in the human were muted compared with those in the rat. Nevertheless, phthalate effects on germ cells have potential implications for the next generation, which merits further study. Our results indicate that the rat is a human-relevant model in which to explore the mechanisms for germ cell effects.<br>**Method:** We used immunohistochemistry, immunofluorescence, and quantitative real-time polymerase chain reaction to examine Sertoli and germ cell markers on rat testes and human fetal testis xenografts after exposure to vehicle or di(n-**butyl) phthalate** (DBP). Our study included analysis of germ cell differentiation markers, proliferation markers, and cell adhesion proteins.<br>**Results:** In both rat and human fetal testes, DBP exposure induced similar germ cell effects, namely, germ cell loss (predominantly undifferentiated), induction of multinucleated gonocytes (MNGs), and aggregation of differentiated germ cells, although the latter occurred rarely in the human testes. The mechanism for germ | van den Driesche et al, 2015 |

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| cell aggregation and MNG induction appears to be loss of Sertoli cell-germ cell membrane adhesion, probably due to Sertoli cell microfilament redistribution. | |
| **Type/Objective:** DBP and its major metabolite, monobutyl phthalate (MBP), change steroid biosynthesis and impair male reproductive function. However, the regulatory mechanism underlying the steroid biosynthesis disruption by MBP is still unclear.<br>**Conclusion:** This study reveals an important and novel mechanism whereby SF-1 and GATA-4 may regulate StAR during MBP-induced steroidogenesis disruption.<br>**Method:** We analyzed the progesterone production, steroidogenic acute regulatory protein (StAR) mRNA, protein expression, and DNA-binding affinity of transcription factors (SF-1 and GATA-4).<br>**Results:** Our results reveal that MBP inhibited progesterone production. At the same time, StAR mRNA and protein were decreased after MBP exposure. Furthermore, electrophoretic mobility shift assay showed that DNA-binding affinity of transcription factors (SF-1 and GATA-4) was decreased in a dose-dependent manner after MBP treatments. Western blot tests next confirmed that protein of SF-1 was decreased, but GATA-4 protein was unchanged. However, phosphorylated GATA-4 protein was decreased with 800 μM of MBP. | Hu et al, 2015 |

**BACKGROUND INFORMATION FOR TABLE 37A: ANIMAL STUDIES ON EFFECTS ON OR VIA LACTATION**

| Summary of Study | Reference |
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| **Type/Objective:** To investigate the neurobehavioral effects of DBP on rodent offspring following in utero and lactational exposure<br>**Conclusion:** The dose level of DBP in the present study produced a few adverse effects on the neurobehavioral parameters, and it may alter cognitive abilities of the male rodent.<br>**Method:** Pregnant Wistar rats were treated with DBP (0, 0.037, 0.111, 0.333 and 1% in the diet) from GD 6 to PND 28, and selected developmental and neurobehavioral parameters of the offspring were measured.<br>**Results:** There were no significant effects of DBP on body weight gain of the dams during GD 6-20 or on the pups' ages of pinna detachment, incisor eruption or eye opening. Exposure to 1% DBP prolonged gestation period, decreased body weight in both male and female pups, depressed surface righting (PND 7) in male pups, shortened forepaw grip time (PND 10), enhanced spatial learning and reference memory (PND 35) in male pups. Exposure to 0.037% DBP also shortened forepaw grip time (PND 10), but inhibited spatial learning and reference memory in male pups. Sex x treatment effects were found in forepaw grip time (PND 10), spatial learning and reference memory, and the male pups appeared to be more susceptible than the females. However, all levels of DBP exposure did not significantly alter surface righting (PND 4), air righting (PND 16), negative geotaxis (PND 4 or 7), cliff avoidance (PND 7) or open field behavior (PND 28) in either sex. | Li Y et al, 2009 |
| **Type/Objective:** Effects of DBP on maze performance in male rats were evaluated by spatial learning tasks; the effects of DBP on the expression of brain-derived neurotrophic factor (BDNF) were also analyzed in both mRNA and mature protein levels in the hippocampus, with intent to investigate the possible mechanism underlying the behavioral findings.<br>**Conclusion:** Our results suggest that developmental treatment with high-dose DBP improves spatial memory in male rats, and this effect may be related to an increase in BDNF expression in the hippocampus in a p-CREB independent route.<br>**Method:** Pregnant Wistar rats were treated orally by gavage with 0, 25, 75, 225 and 675mgDBP/kgBW/day from gestational day (GD) 6 to postnatal day (PND) 21, and then the weaned offspring continued receiving the same treatment till PND 28.<br>**Results:** We found that male pups treated with high-dose DBP showed enhancement in spatial acquisition in a Morris water maze during PNDs 30-33, and displayed better retention of spatial memory in a probe trial after a reverse trail during PNDs 60-62. Real-time PCR and western blotting analysis of the hippocampus from DBP-treated male rats on PND 21 revealed an increase in BDNF expression, compared to the vehicle-matched control. BDNF variant III, a | Li Y et al, 2010 |

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| transcription promoted by active CREB (i.e. p-CREB), as well as the immunocontent of p-CREB, was scarcely altered by the treatment. | |
| **Type/Objective:** To evaluate the effects of DBP exposure during fetal and lactational periods on the male adult rat prostate<br>**Conclusion:** Results showed that DBP could play a role in proliferative and inflammatory disorders of the rat prostate.<br>**Method:** Pregnant females were distributed into two experimental groups: Control (C) and Treated (T). The females of the T group received DBP (100mg/kg, by gavage) from gestation day 12 to postnatal day 21, while C rats received the vehicle (corn oil). In adulthood (90 days old), the animals were euthanized. The serum and testicular testosterone levels were measured. Ventral prostate was removed and weighed. Distal segment fragments of the ventral prostate were fixed and processed for histochemistry and immunohistochemistry to detect androgen receptor (AR) and Ki67 antigens. Protein extraction from ventral prostate fragments was performed for AR immunoblotting and Gelatin zymography for MMP-2 and MMP-9 (MMP, metalloproteinase). Stereological and histopathological analyses were also performed.<br>**Results:** Serum and testicular testosterone levels and prostate weight were comparable between groups. In the T group the relative proportions (%) of epithelial (C=32.86; T=42.04*) and stromal (C=21.61; T=27.88*) compartments were increased, while the luminal compartment was decreased (C=45.54; T=30.08*), *p<0.05. InT, disseminated inflammatory infiltrate in the stroma, associated or not with epithelial dysplasia and PIN (Prostatic Intraepithelial Neoplasia), was observed. Increases in AR expression, proliferation index and metalloproteinase 9 (MMP-9) activity were noted in T animals. In some T animals, collagen fibrils accumulated adjacent to the epithelium. | Scarano et al, 2009 |
| **Type/Objective:** To investigate effects, with emphasis on the epididymis, of in utero and lactational exposure to 100 mg DBP/kg/d in adult male rat offspring<br>**Conclusion:** These results showed that fetal testes were affected by DBP as evidenced by testicular histopathologic alterations, but reproductive parameters and epididymal structure/function were not significantly altered in the adult animals exposed to 100 mg/kg DBP in utero and during lactation.<br>**Method:** The fetal testis histopathology was also determined. The selected endpoints included reproductive organ weights, sperm motility and morphology, sperm epididymal transit time, sperm quantity in the testis and epididymis, hormonal status, fetal testis and epididymal histopathology and stereology, and androgen receptor (AR), aquaporin 9 (AQP9), and Ki-67 immunoreactivities. Pregnant females were divided into two groups: control (C) and treated (T). The treated females received DBP (100 mg/kg/d, by gavage) from gestation day (GD) 12 to postnatal day (PND) 21, while control dams received the vehicle. Some pregnant dams were killed by decapitation on GD20, and testes from male fetuses were collected for histopathogy. Male rats from other dams were killed at PND 90.<br>**Results:** Fetal testes from treated group showed Leydig-cell clusters, presence of multinucleated germinative cells, and increase of the interstitial component. Testosterone levels and reproductive organ weights were similar between the treated and control adult groups. DBP treatment did not markedly affect relative proportions of epithelial, stromal, or luminal compartments in the epididymis; sperm counts in the testis and epididymis; sperm transit time; or sperm morphology and motility in adult rats. The AR and AQP9 immunoreactivities and proliferation index were similar for the two groups. | Scarano et al, 2010 |

**BACKGROUND INFORMATION FOR TABLE 37B: HUMAN DATA ON EFFECTS ON OR VIA LACTATION**

| Summary of Study | Reference |
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| **Type/Objective:** DBP has been identified in human breast milk in concentrations ranging from 10 to 51 µg/kg (Gruber et al., 1998; Bruns-Weller and Pfordt, 2000).<br>**Conclusion:** The exposure via breast milk for infants thus varies between 1.2 and 6 µg DBP/kg bw/day.<br>**Method:** The exposure to babies was calculated according to the WHO (1998). For the first three months in life, an infant consumes an average of 120 grams per day of human milk per kilogram of body weight. After three months of age, the volume consumed per unit weight of the infant decreases with increasing age. By multiplying the concentration (given as mg/kg or mg/l) of a particular substance in whole breast milk by a factor of 0.12, the approximate daily intake of the substance in mg/kg bw/day can be estimated. If the concentration is given in mg/kg milk fat and the milk fat content is not reported, it is assumed that the average fat content of the milk is 3.5%.<br>**Results:** Based on the concentrations found, the exposure to DBP via breast milk can be calculated as follows:<br>minimum: 10 µg DBP/kg milk = 100.120 = 1.2 µg DBP/kg bw/day<br>maximum: 51 µg DBP/kg milk = 51.0.120 = 6 µg DBP/kg bw/day. | EC, 2003 |
| **Type/Objective:** Analysis of breast milk samples for phthalate monoesters and serum samples from boys with cryptorchidism (failure of testes to descend into the scrotum) for gonadotropins, sex-hormone binding globulin (SHBG), testosterone, and inhibin B.<br>**Conclusion:** No association between phthalate monoesters and cryptorchidism was found, but MBP was associated with SHBG and LH:free testosterone ratio and negatively associated with free testosterone. Authors considered these and results with other monoesters to be in accordance with rodent data and suggested that human Leydig cell development and function may also be vulnerable to perinatal exposure to some **phthalates.**<br>**Method:** We obtained biologic samples from a prospective Danish-Finnish cohort study on cryptorchidism from 1997 to 2001. We analyzed individual breast **milk** samples collected as additive aliquots 1-3 months postnatally (n = 130; 62 cryptorchid/68 healthy boys) for **phthalate** monoesters [mono-methyl **phthalate** (mMP), mono-ethyl **phthalate** (mEP), mono-n-butyl **phthalate** (mBP), mono-benzyl **phthalate** (mBzP), mono-2-ethylhexyl **phthalate** (mEHP), mono-isononyl **phthalate** (miNP)]. We analyzed serum samples (obtained in 74% of all boys) for gonadotropins, sex-hormone binding globulin (SHBG), testosterone, and inhibin B.<br>**Results:** All **phthalate** monoesters were found in breast **milk** with large variations [medians (minimum-maximum)]: mMP 0.10 (< 0.01-5.53 microg/L), | Main et al, 2006 |

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| mEP 0.95 (0.07-41.4 microg/L), mBP 9.6 (0.6-10,900 microg/L), mBzP 1.2 (0.2-26 microg/L), mEHP 11 (1.5-1,410 microg/L), miNP 95 (27-469 microg/L). Finnish breast **milk** had higher concentrations of mBP, mBzP, mEHP, and Danish breast **milk** had higher values for miNP (p = 0.0001-0.056). No association was found between **phthalate** monoester levels and cryptorchidism. However, mEP and mBP showed positive correlations with SHBG (r = 0.323, p = 0.002 and r = 0.272, p = 0.01, respectively); mMP, mEP, and mBP with LH:free testosterone ratio (r = 0.21-0.323, p = 0.002-0.044) and miNP with luteinizing hormone (r = 0.243, p = 0.019). mBP was negatively correlated with free testosterone (r = -0.22, p = 0.033). Other **phthalate** monoesters showed similar but nonsignificant tendencies. | |

## BACKGROUND INFORMATION FOR TABLE 37C: OTHER STUDIES RELEVANT FOR EFFECTS ON OR VIA LACTATION

No information was found.

**TABLE 38A: SUMMARY TABLE OF ANIMAL STUDIES RELEVANT FOR STOT WITH SINGLE EXPOSURE**

| Summary of Study | Reference |
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| **Type/Objective:** Di-iso-butyl phthalate (DiBP) is used as a substitute for DBP. The effects of DiBP on testes in prepubertal rodents still remain to be obscure. Testicular toxicity of DiBP was investigated in 21-day-old Sprague-Dawley rats and C57BL/6N mice, using with in situ TUNEL method.<br>**Conclusion:** DiBP can induce testicular atrophy in rats due to the increase of TUNEL-positive spermatogenic cells in both acute and 7-day exposures.<br>**Method:** For an acute exposure experiment, animals were once given DiBP at various concentrations by oral gavage. In a second study, they were daily given DiBP at various concentrations for consecutive 7 days. Controls were treated with corn oil under the same condition. For a recovery experiment, rats were once given DiBP (1000 mg/kg), and were sacrificed at day 1 to 8 after administration.<br>**Results:** The disorder of vimentin filaments in Sertoli cells after daily administration of DiBP (500 mg/kg) for consecutive 7 days in rats also identified by immunohistochemistry using anti-vimentin antibody. As a result, the present study demonstrated that DiBP can induce testicular atrophy in rats due to the increase of TUNEL-positive spermatogenic cells in both acute and subchronic exposure experiments. At the same time, the disorder of vimentin filaments in Sertoli cells was recognized. However, no such damages could be found in mouse testis. For the recovery experiment, the testis weight and testicular morphology returned to normal at day 6 after administration. In conclusion, the present study indicates that DiBP causes the significant increase of TUNEL-positive spermatogenic cells and the disorder of vimentin filaments in Sertoli cells in rats and that DiBP shows a species-specific toxicity. | Zhu et al, 2010 |
| **Type/Objective:** A study on effects of DBP with acute exposure of rats<br>**Conclusion:** These results lead us to the postulation that a single administration of DBP to prepubertal rats delays maturation of spermatogenic cells, even after completion of first wave of spermatogenesis.<br>**Method:** Morphological alterations in seminiferous tubules caused by single administration of DBP in 3-week-old rats were investigated throughout the first wave of spermatogenesis.<br>**Results:** Single administration of DBP (500 mg/kg) showed progressive detachment and displacement of spermatogenic cells and disappearance of tubular lumen at 3h after treatment, and then showed thin seminiferous epithelia and wide tubular lumen at day 1 (D1). At D1, quite significant numbers of apoptotic spermatogenic cells were detected, and then they gradually decreased in accordance with the passage of time. In contrast, the testes revealed lower weight gain, even after completion of first wave of spermatogenesis in the DBP-treated group, compared to the control. In order to clarify whether spermatogenic cells differentiate into mature spermatids in the DBP-treated rats, | Alam et al, 2010a |

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| immunohistochemical staining for Hsc 70t, a specific marker for elongate spermatids, was carried out. As a result, the decrease in mature spermatids in the DBP-treated testes, compared to the control, was demonstrated. For example, at D20 (41-day-old) after treatment, the most advanced spermatids in the tubules from rats in the DBP-treated groups were steps 2-4, while those of the control were steps 12-13. Moreover, in some tubules, pachytene spermatocytes were the most advanced spermatogenic cell. At D30 (51-day-old) after treatment, maturation of spermatogenic cells in the DBP-treated rats proceeded further, and the most advanced spermatids in tubules were steps 8-9, while those of the control were steps 15-19. | |
| **Type/Objective:** Previous studies have revealed that DBP induces spermatogenic cell apoptosis, although its mechanism is not yet clear. The present study describes that disruption of Sertoli cell vimentin filaments by DBP administration may relate to spermatogenic cell apoptosis.<br>**Conclusion:** These in vivo and in vitro experiments indicate that DBP-induced collapse of Sertoli cell vimentin filaments may lead to detachment of spermatogenic cells, and then detached cells may undergo apoptosis because of loss of the support and nurture provided by Sertoli cells.<br>**Method:** DBP was given once orally to rats at a dose of 500 mg/kg.<br>**Results:** The present histopathological study revealed that a single oral administration of 500 mg/kg DBP caused progressive detachment and displacement of spermatogenic cells away from the seminiferous epithelium and sloughing of them into the lumen. Degenerative spermatogenic cells characterized by chromatin condensation were frequently observed in DBP-treated rats. Ultrastructurally, the degenerative spermatogenic cells were separated from their neighbours, and a collapse of Sertoli cell vimentin filaments was recognized in DBP-treated rats. Sertoli cell cultures showed the increased number and size of vacuoles in their cytoplasm. In agreement with the in vivo experiment, vimentin filaments clearly showed a gradual collapse in DBP-exposed Sertoli cells in vitro. | Alam et al, 2010b |

## TABLE 38B: SUMMARY TABLE OF HUMAN DATA RELEVANT FOR STOT WITH SINGLE EXPOSURE

No information was found.

## TABLE 38C: SUMMARY TABLE OF OTHER STUDIES RELEVANT FOR STOT WITH SINGLE EXPOSURE

No information was found.

**TABLE 39A: SUMMARY TABLE OF ANIMAL STUDIES RELEVANT FOR STOT WITH REPEATED EXPOSURES**

| Summary of Study | Reference |
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| **Type/Objective:** Limited dietary study in mice<br>**Conclusion:** Kidneys and liver were affected by the exposure to DBP.<br>**Method:** 0.25 or 2.5% DBP in diet (~ 500 and 5,000 mg/kg bw) was administered for 86 or 90 days.<br>**Results:** Remarkable vacuolar degeneration and necrosis of single cells in the liver, and cysts and degeneration of epithelial cells in the renal tubules were observed in the high-dose group. In the low-dose group, histological changes were slight in the liver and kidneys but degeneration of parenchyma was observed. | Ota et al, 1973; 1974, as summarized in EC, 2003 |
| **Type/Objective:** NTP conducted a 14-day dietary range-finding study with DBP in CD Sprague-Dawley rats.<br>**Conclusion:** No animals died and clinical signs were normal. Food consumption and body weights were affected at the higher doses. The results were used to select the exposures of 0, 1000, 5000, or 10,000 ppm in the continuous breeding study (NTP, 1995).<br>**Method:** There were 8 rats/ sex/ group; animals were 10 weeks of age. Dibutyl phthalate was administered in the feed at 0, 1000, 5000, 10,000, 15,000, or 20,000 ppm (exposures in males were 0, 70, 340, 650, 910, or 1190 mg/kg-day and in females were 0, 70, 350, 700, 930, or 1150 mg/kg-day). The animals were monitored for mortality, clinical signs of toxicity, food consumption, and body weight.<br>**Results:** No animals died and there were no clinical signs of toxicity reported during the study. Food consumption over the 14-day study was decreased at the two highest exposures (19 and 21% in males and 13 and 18% in females, respectively). Body weights were reduced in males at the two highest exposures (10 and 8%, respectively) and in females at the three highest exposures (10, 7, and 12%, respectively). | NTP, 1991 |
| **Type/Objective:** 3-month dietary study following OECD Guideline 408,<br>**Conclusion:** Wistar rat<br>**Method:** Guideline study using Wistar rats<br>**Results:** A dose of 152 mg/kg bw appeared to be the NOAEL. At the next higher dose-level of 752 mg/kg bw changes in hematological (decreased haemoglobin- and haematocrit-values and decreased erythrocyte counts) and clinical chemical parameters (decreased triglyceride levels, increased serum glucose and albumin levels), a statistically significant increase in the activity of cyanide-insensitive palmitoyl-CoA oxidase (PCoA; is an indicator for peroxisomal proliferation), a statistically significant decrease in T3 and statistically significant increases in liver and kidney weights were observed. Histopathology showed decreased or missing lipid deposition in hepatocytes at 752 mg/kg bw. Neurofunctional tests did not show abnormalities at any dose-level. No effect on the testes was observed | Schilling et al, 1992, as summarized in EC, 2003 |

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| in this study. | |
| **Type/Objective:** A 13-week evaluation by NTP of the toxicity of DBP in male and female F344 rats. DBP was given in the diet.<br>**Conclusion:**. Liver and testes were both affected. The NOAEL for effects in the testis is 359 mg/kgday (5000 ppm), and the LOAEL is 720 mg/kg-day (10,000 ppm). The NOAEL for effects in the liver is 176 mg/kg-day (2500 ppm), and the LOAEL is 359 mg/kg-day (5000 ppm).<br>**Method:** Rats (n = 10 of each sex in each group) received dibutyl phthalate in the diet at 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm (equivalent to 0, 176, 359, 720, 1540, or 2964 mg/kg-day in males and 0, 177, 356, 712, 1413, or 2943 in females).<br>**Results:** No deaths occurred. Markedly reduced final mean body weights were observed in males and females in the 40,000 ppm groups (a decrease of 45 and 73%, respectively). Increases in relative liver weight were observed in males that received 5000 ppm or greater (of 18, 32, 54, and 70%, respectively) and in females that received 10,000 ppm or greater (increases of 11, 25, and 78%, respectively). Testis and epididymal weights of males in the 20,000- and 40,000-ppm groups were lower than those of the controls. Hypocholesterolemia was observed in male and female rats receiving 20,000 or 40,000 ppm, and hypotriglyceridemia was detected in males in all exposed groups and in females receiving 10,000 ppm or greater. Elevations in alkaline phosphatase activity and bile acid concentration in male and female rats were considered indicative of cholestasis.<br><br>Morphologic evaluation confirmed the toxicity of dibutyl phthalate to the liver and testis of rats. Microscopic examination of the liver revealed hepatocellular cytoplasmic alterations, consistent with glycogen depletion, in male and female rats receiving 10,000 ppm or greater. In the liver of rats in the 40,000-ppm groups, small, fine eosinophilic granules were also observed in the cytoplasm of hepatocytes. Ultrastructural examination suggested the presence of increased numbers of peroxisomes, and peroxisomal enzyme activity (palmitoyl-CoA oxidase activity) was elevated in the livers of rats administered 5000 ppm or greater. In males, increases of 1.9-, 5.7-, 9.7-, and 13.5-fold, respectively, were observed; in females increases of 1.7-, 2.6-, 11-, and 32.5-fold, respectively, were observed. Lipofuscin accumulation was detected in rats receiving 10,000 ppm or greater.<br><br>Histopathologic examination of the testes revealed degeneration of the germinal epithelium. There was a mild to marked focal lesion in the 10,000- and 20,000-ppm groups and a marked diffuse lesion in all males in the 40,000 ppm group resulting in almost complete loss of the germinal epithelium at 40,000 ppm. Testicular zinc concentrations were lower in the 20,000and 40,000-ppm groups than in the controls. Serum testosterone values were also lower at these concentrations than in controls. Spermatogenesis was evaluated in males in the 0-, 2500-, 10,000-, and 20,000-ppm groups. At 20,000 ppm, spermatid heads per testis and per gram testis, epididymal spermatozoal motility, and the number of | NTP, 1995 |

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| epididymal spermatozoa per gram epididymis were lower than in the controls. All of these findings are consistent with the marked loss of germinal epithelium at this exposure. | |
| **Type/Objective:** A 13-week dietary study with DBP was conducted in B6C3F1 mice by NTP.<br>**Conclusion:** Hepatocellular cytoplasmic alterations were seen and mean body weight was lower at high doses. The NOAEL is 5000 ppm (equivalent to 812 mg/kg-day in males and 971 mg/kg-day in females) and the LOAEL is 10,000 ppm (equivalent to1601 mg/kg-day in males and 2137 mg/kg-day in females).<br>**Method:** Mice received 0, 1250, 2500, 5000, 10,000, or 20,000 ppm dibutyl phthalate in feed (equivalent to 0, 163, 353, 812, 1601, or 3689 mg/kg-day in males and 0, 238, 486, 971, 2137, or 4278 mg/kg-day in females).<br>**Results:** No deaths occurred during this study. Mean body weights were decreased by 13% in males and 13% in females at 20,000 ppm. An increase in relative liver weight was observed in males and females at 10,000 ppm or greater (an increase of 16% and 38% in males, respectively, and 19% and 52% in females, respectively). Although no gross lesions were observed at necropsy, microscopic examination revealed hepatocellular cytoplasmic alterations, consistent with glycogen depletion, in male mice receiving 10,000 or 20,000 ppm and female mice receiving 20,000 ppm. Small, fine eosinophilic granules were also observed in the cytoplasm of hepatocytes in males and females in the 20,000 ppm groups. Lipofuscin accumulation in the liver was detected in males and females receiving 10,000 ppm or greater. | NTP, 1995 |
| **Type/Objective:** Inhalation experiment in Wistar rats (5/sex/group) according to OECD Guideline No. 412 and (for clinical and neurofunctional examinations and pathology) to OECD No. 407<br>Conclusion:<br>**Method:** Head-nose exposure 6 hours/day, 5 days/week, for 4 weeks, to measured concentrations of 0, 1.18, 5.57, 49.3 or 509 mg DBP (purity 99.8%)/$m^3$ of air as liquid aerosol (MMAD = 1.5-1.9 µm; GSD ~ 2).<br>**Results:** No systemic effects, including neurotoxic effects, were observed up to and including the highest exposure concentration of 509 mg/m3. Therefore, the NOAEC for systemic effects in this study is 509 mg/$m^3$, the highest concentration tested. For local effects in the upper respiratory tract no NOAEC can be determined in this study since adverse local effects were observed even at the lowest exposure concentration of 1.18 mg/$m^3$. Therefore 1.18 mg/m3 is a LOAEC for local effects in the upper respiratory tract in this study. | Gamer et al., 2000 , as Summarized in EC, 2003 |

**TABLE 39B: SUMMARY TABLE OF HUMAN DATA RELEVANT FOR STOT WITH REPEATED EXPOSURES**

| Summary of Study | Reference |
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| **Type/Objective:** The aim of this study was to investigate the impact of phthalates on symptoms of ADHD in school-age children.<br>**Conclusion:** The present study showed a strong positive association between phthalate metabolites in urine and symptoms of ADHD among school-age children.<br>**Method:** A cross-sectional examination of urine phthalate concentrations was performed, and scores on measures of ADHD symptoms and neuropsychological dysfunction with regard to attention and impulsivity were obtained from 261 Korean children, age 8-11 years.<br>**Results:** Mono-2-ethylheyl phthalate (MEHP) and mono-2-ethyl-5-oxohexylphthalate (MEOP) for metabolites of Di-2-ethylhexylphthalate (DEHP) and mono-n-butyl phthalate (MNBP) for metabolites of dibutyl phthalate (DBP) were measured in urine samples. The mean concentrations of MEHP, MEOP, and MNBP were 34.0 microg/dL (SD = 36.3; range: 2.1-386.7), 23.4 microg/dL (SD = 23.0; range: .75-244.8), and 46.7 microg/L (SD = 21.4; range: 13.2-159.3), respectively. After adjustment for covariates, teacher-rated ADHD scores were significantly associated with DEHP metabolites but not with DBP metabolites. We also found significant relationships between the urine concentrations of metabolites for DBP and the number of omission and commission errors in continuous performance tests (CPT) after adjustment for covariates. | Kim BN et al, 2009 |
| **Type/Objective:** To investigate the associations of hormone circulation with phthalate exposure in adult men.<br>**Conclusion:** Serum PRL is suggested to be positively associated with both DBP and DEHP exposure in adult men.<br>**Method:** Semen and serum samples were collected from 118 men who were suspected of infertility. Phthalate diesters including dibutyl phthalate (DBP) and diethylhexyl phthalate (DEHP) in both semen and serum samples were measured, along with serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), estradiol (E(2)) and prolactin (PRL).<br>**Results:** Serum PRL was positively associated with serum DBP and DEHP and semen DEHP in all models of Spearman correlation, linear regression and binary logistic regression. In linear regression models adjusted for potential confounders and excluding subjects with undetectable phthalates, a 10-fold increase in semen DEHP was associated with a 23% increase in serum PRL, as well as a 26% increase in serum DBP and a 20% increase in serum DEHP. In logistic regression models all subjects demonstrated a dose-response relationship between above reference value PRL and semen DEHP (odds ratio per tertile adjusted for potential confounders = 1.0, 1.70, 3.50; P for trend = 0.01), and serum DBP (1.0, 1.10, 2.62; P for trend = 0.04), and serum DEHP (1.0, 1.46, 4.69; P for trend < 0.01). A positive correlation between serum estradiol and semen DEHP (linear regression), and an inverse correlation between semen DBP and serum testosterone and T:E(2) ratio (Spearman correlation) were also established. | Li S et al, 2011 |

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| **Type/Objective:** To examine the association of prenatal exposure to bisphenol A and select common phthalates with infant neurobehavior measured at 5 weeks.<br>**Conclusion:** The association between prenatal phthalate exposure and infant neurobehavior differed by type of phthalate and was evident only with exposure measured at 26w. Prenatal exposure to DBP was associated with improved behavioral organization in 5-week-old infants. Prenatal exposure to DEHP was associated with nonoptimal reflexes in male infants. There was no evidence of an association between prenatal BPA exposure and infant neurobehavior.<br>**Method:** We compared the concentration of maternal urinary metabolites of bisphenol A and phthalates at two distinct time points in pregnancy (16w, 26w) with scores on the NICU Network Neurobehavioral Scale (NNNS) at 5 weeks of age in a cohort of 350 mother/infant pairs.<br>**Results:** Prenatal exposure to BPA was not significantly associated with neurobehavioral outcomes at 5 weeks. Significant associations between prenatal exposure to measured phthalates and infant neurobehavioral outcomes differed by type of phthalate and were only seen with exposure measured at 26 weeks. Higher total di-butyl phthalate (DBP) metabolites at 26w were associated with improved behavioral organization evidenced by decreased arousal (p=.04), increased self-regulation (p=.052), and decreased handling (p=.02). In males, higher total di-2-ethylhexyl phthalate (DEHP) metabolites at 26w were associated with more nonoptimal reflexes (p=.02). | Yolton et al, 2011 |
| **Type/Objective:** Limited animal, in vitro, and human studies have reported that exposure to phthalates or bisphenol A (BPA) may affect thyroid signaling. We explored the cross-sectional relationship between urinary concentrations of metabolites of di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), and BPA with a panel of serum thyroid measures among a representative sample of U.S. adults and adolescents.<br>**Conclusion:** These results support previous reports of associations between phthalates-and possibly BPA--and altered thyroid hormones. More detailed studies are needed to determine the temporal relationships and potential clinical and public health implications of these associations.<br>**Method:** We analyzed data on urinary biomarkers of exposure to phthalates and BPA, serum thyroid measures, and important covariates from 1,346 adults (ages ≥ 20 years) and 329 adolescents (ages 12-19 years) from the National Health and Nutrition Examination Survey (NHANES) 2007-2008 using multivariable linear regression.<br>**Results:** Among adults, we observed significant inverse relationships between urinary DEHP metabolites and total thyroxine (T4), free T4, total triiodothyronine (T3), and thyroglobulin, and positive relationships with thyroid-stimulating hormone (TSH). The strongest and most consistent relationships involved total T4, where adjusted regression coefficients for quintiles of oxidative DEHP metabolites displayed monotonic dose-dependent decreases in total T4 (p-value for trend < 0.0001). Suggestive inverse relationships between urinary BPA and total T4 and TSH were also observed. Conversely, among adolescents, we observed significant positive relationships between DEHP metabolites and total T3. Mono(3-carboxypropyl) phthalate, a secondary metabolite of both DBP and di-n-octyl phthalate, was associated with several thyroid measures in both age groups, whereas other DBP metabolites were not associated with thyroid measures. | Meeker and Ferguson, 2011 |
| **Type/Objective:** This study investigates the association between urinary phthalate metabolite levels and attention deficit disorder (ADD), learning disability (LD), and co-occurrence of ADD and LD in 6-15-year-old children.<br>**Conclusion:** We found cross-sectional evidence that certain phthalates are associated with increased odds of ADD and both ADD and LD.<br>**Method:** We used cross-sectional data from the National Health and Nutrition Examination Survey (NHANES, 2001-2004). Phthalate metabolites with ≥75% detection | Chopra et al, 2013 |

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| in urine samples were examined. The study population comprised 1493 children with parent-reported information on ADD or LD diagnosis and phthalate concentrations in urine. Phthalate concentrations were creatinine-adjusted and log10-transformed for analysis. All models controlled for child sex, age, race, household income, blood lead, and maternal smoking during pregnancy.<br>**Results:** There were 112 ADD cases, 173 LD cases, and 56 ADD and LD cases in the sample. After adjusting for potential confounders, we found increased odds of ADD with increasing urinary concentration of di-2-ethylhexyl phthalates (OR: 2.1; 95% CI: 1.1, 3.9) and high molecular weight phthalates (OR: 2.7; 95% CI: 1.2, 6.1). In addition, dibutyl phthalates (OR: 3.3; 95% CI: 0.9, 12.7) and high molecular weight phthalates (OR: 3.7; 95% CI: 0.9, 14.8) were marginally associated with increased odds of co-occurring ADD and LD. We did not find associations for any phthalate and LD alone. We observed stronger associations between phthalates and ADD and both ADD and LD in girls than boys in some models. | |
| **Type/Objective:** The hypothesis that exposure to phthalates may increase kisspeptin secretion and thereby cause early-onset puberty is unexplored.<br>**Conclusion:** Kisspeptin may promote the onset of puberty in girls who are exposed to a high level of phthalates, especially DBP. Our study suggests that the early onset of puberty is related to increased kisspeptin secretion.<br>**Method:** This case-control study ran from 2006 to 2009. We enrolled 104 girls. Girls in the central precocious puberty (CPP) (case) group were recruited from a pediatric endocrinology policlinic in Taiwan; prepubescent controls were recruited from local elementary schools and all were categorized based on a pediatrician's diagnosis. The physical characteristics of puberty were assessed and levels of LH, FSH estradiol and kisspeptin-54 in blood samples were evaluated using radioimmunoassay. Reversed-phase high-performance liquid chromatography-tandem mass spectrometry was used to analyze seven urinary phthalate metabolites. Non-parametric analyses, trend tests and linear regressions were performed on the data.<br>**Results:** All seven urinary phthalate metabolites in the CPP group were significantly ($P < 0.05$) higher than in prepubescent controls. Serum kisspeptin-54 levels were higher ($P = 0.022$) in the CPP group than controls and were still significantly higher after adjusting for age ($P = 0.03$). There was a significant increasing trend ($P(trend) = 0.005$) between levels of kisspeptin and the stages of puberty. The concentration of kisspeptin-54 did not change in girls treated with leuprorelin acetate. There was a significant positive correlation between kisspeptin-54 and urinary MBP (ng/ml: $R(2) = 0.251$, $P < 0.001$; μg/g-creatinine: $R(2) = 0.109$, $P = 0.024$). However, the study duration was short and the sample size relatively small; therefore, we were unable to collect sufficient evidence to support the temporality between exposure to phthalates and the subsequent occurrence of PP. | Chen et al, 2013 |
| **Type/Objective:** To investigate the relationship between urinary phthalate metabolite concentrations and the risk of a hormonally-driven disease, endometriosis, in reproductive-age women.<br>**Conclusion:** Exposure to select phthalates is ubiquitous among female enrollees of a large healthcare system in the U.S. Pacific Northwest. The findings from our study suggest that phthalates may alter the risk of a hormonally-mediated disease among reproductive-age women.<br>**Method:** We used data from a population-based case-control study of endometriosis, conducted among female enrollees of a large healthcare system in the U.S. Pacific Northwest. We measured urinary phthalate metabolite concentrations on incident, surgically-confirmed cases (n=92) diagnosed between 1996 and 2001 and population-based controls (n=195). Odds ratios (OR), and 95% confidence intervals (CI) were estimated using unconditional logistic regression, adjusting for urinary creatinine | Upson et a, 2013 |

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| concentrations, age, and reference year.<br>**Results:** The majority of women in our study had detectable concentrations of phthalate metabolites. We observed a strong inverse association between urinary mono-(2-ethyl-5-hexyl) phthalate (MEHP) concentration and endometriosis risk, particularly when comparing the fourth and first MEHP quartiles (aOR 0.3, 95% CI: 0.1-0.7). Our data suggested an inverse association between endometriosis and urinary concentrations of other di-2-ethylhexyl phthalate (DEHP) metabolites (mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)) and ∑DEHP, however, the confidence intervals include the null. Our data also suggested increased endometriosis risk with greater urinary concentrations of mono-benzyl phthalate (MBzP) and mono-ethyl phthalate (MEP), although the associations were not statistically significant. | |
| **Type/Objective:** Endocrine disruptors that mimic natural hormones and inhibit the action of hormones have recently attracted attention as one of the main cause of precocious puberty. In this study, the levels of 7 EDCs and 3 isoflavones that exhibit estrogen-like actions were measured in the plasma of precocious puberty patients and compared to control subjects to determine if there is an association between the onset of precocious puberty and the levels of EDCs in the plasma.<br>**Conclusion:** The results suggest that these six substances (MBP, t-OP, n-NP, daidzein, equol, and genistein) have an effect on precocious puberty.<br>**Method:** EDCs examined in this study were bisphenol-A (BPA), di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), mono(2-ethylhexyl) phthalate (MEHP), monobutyl phthalate (MBP), n-nonyl phenol (n-NP), and t-octylphenol (t-OP), and whereas the isoflavones were equol, genistein, and diadzein.<br>**Results:** The level of MBP in the plasma of patients was 1.3 times higher than that of the controls. The levels of t-OP and n-NP in the plasma of patients were respectively 1.15 and 1.2 times higher than those of the control group. Finally, the levels of the diadzein, equol and genistein were 1.37, 1.3 and 2.67 times higher than those of the control group, and genistein showed a statistically meaningful result (P = 0.0008). | Yum et al, 2013 |

**TABLE 39C: SUMMARY TABLE OF OTHER STUDIES RELEVANT FOR STOT WITH REPEATED EXPOSURES**

No relevant references were found other than those related to reproductive effects.

## BACKGROUND INFORMATION FOR TABLE 41: RAPID DEGRADABILITY

| Summary of Study | Reference |
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| **Type/Objective:** Degradation of phthalate esters in mangrove sediments<br>**Conclusion:** Our results showed that Bacillus sp. was the dominant bacteria in the process of PAE aerobic degradation in the mangrove sediments.<br>**Method:** we investigated the microbial degradation of the phthalate esters (PAEs) dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP), and change in microbial communities in mangrove sediment collected from 5 sampling sites along the Tanshui River in Taiwan.<br>**Results:** Aerobic degradation half-lives (t(1/2)) of DBP and DEHP ranged from 1.6 to 2.9 d and 5.0 to 8.3d, respectively. The addition of yeast extract (5mg/L), hydrogen peroxide (1mg/L), brij 35 (91 μM), humic acid (0.5 g/L), cellulose (0.96 mg/L), and sodium chloride (1%) enhanced PAE aerobic degradation. Sediment samples were separated into fractions with various particle size ranges from 0.1-0.45 to 500-2000 μm. Sediment fractions with smaller particle sizes demonstrated higher PAE biodegradation rates. Of the microorganism strains isolated from the mangrove sediment, strains J2, J4, and J8 (all identified as Bacillus sp.) expressed the best biodegrading ability. | Yuan et al, 2010 |

### Addendum to Table 41 – Rapid Degradability Studies Not Useful for Classification

| Summary of Studies | References |
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| **Type/Objective:** A wide range of environmental and chemical factors influenced the biodegradation of simple and complex phthalic acid esters in an **aquatic** environment. The length and configuration of the alkyl phthalate diester significantly affected the primary biodegradation of di-n-butyl (DBP), di-2-ethylhexyl (DEHP), diisooctyl (DIOP) and diisononyl (DNIP) phthalate.<br>**Conclusion:** Degradation of DBP was more rapid than branched phthalate esters; 85% of DBP was degraded after 14 days incubation in aerobic sediments at 2°C.<br>**Method and Results:** After 14 days incubation in aerobic sediments at 2°C, < 2% of the branched chain alkyl phthalates, DEHP, DIOP and DINP (at microgram concentrations), were biodegraded, compared with 85% of the linear alkyl DBP. Primary biodegradation of DEHP, DIOP and DINP was significantly greater at high concentrations (> microgram/liter) and high temperatures (> 22°C) in freshwater sediments. Pre-exposure of the sediments to DBP, DEHP, DIOP and DINP did not influence their biodegradation. The addition of organic nutrients significantly affected the primary biodegradation of DBP with varied results, depending on the nutrient, its concentration and the time of addition. Inorganic N or P, alone or in combination, did not influence the degradation of DBP. The complex alkyl phthalate DEHP, in sediments, biodegraded under anaerobic conditions; even though the process was | Johnson et al, 1984 |

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| slow, primary and ultimate degradation did occur. | |
| **Type/Objective:** Degradation of DBP by Psuedomonas sp.<br>**Method and Results:** A psychrotrophic denitrifying Pseudomonas fluorescens was isolated from an unamended subsurface microcosm incubated at 10°C for 43 days. This pseudomonad transformed DBP at 10°C in a chemically defined medium under both aerobic and anaerobic conditions using NO3- as the terminal electron acceptor. Biotransformation of DBP by the pseudomonad appeared to take place only in the stationary and decline phases of growth and was correlated to the amount of biomass. Studies with growth inhibitors and outer membrane permeabilizers suggested that the outer membrane of the cells in the stationary and decline phases was possibly made more permeable to DBP, which would explain the biotransformation pattern. Butanol was produced and utilized by cells that transformed DBP, implying that the side chains were hydrolyzed by means of an esterase. An esterase was detected by staining after isolation by gel electrophoresis. | Chauret et al, 1995 |
| **Type/Objective:** Authors analyze the pollution and degradation characteristics of DBP and DEHP in two kinds of soils collected from non-cultivated, crop, greenhouse, and vegetable fields from the Harbin and Handan Districts, China.<br>**Results:** The results demonstrate that DBP has relatively high residual levels in the soils, ranging from 3.18 to 29.37 mg/kg in fluvo-aquic soils of the Handan District (average 14.06 mg/kg) and 2.75-14.62 mg/kg in black soils of the Harbin District (average 7.60 mg/kg). Residual levels of DEHP reach 1.15-7.99 mg/kg in fluvo-aquic soils of the Handan District (average 4.86 mg/kg) and 0.44-4.20 mg/kg in black soils of the Harbin District (average 2.35 mg/kg). All non-cultivated soils contain the lowest contents of PEs, suggesting that the kinds of pollutants are largely derived from human agricultural activities. Laboratory experiments verify that the degradations of two kinds of PEs are mainly via microbial processes. The microbial populations are higher and reduce more slowly in black soils than those in fluvo-aquic soils. These observations might partially explain the lower levels of residuals and higher degradation rates of PEs pollutants in black soils than those in fluvo-aquic soils. The detection of DBP metabolites indicates that DBP biodegradation might begin by ester hydrolysis to form monobutyl phthalate (MBP) and corresponding alcohol. The MBP then degrades to phthalic acid or butyl benzoate, which might be possibly caused by microbial decarboxylation. The two derivatives of MBP degrade to form protocatechuate through ring cleavage. | Xu et al, 2008 |
| **Type/Objective:** A new small capacity-wide extraction method was proposed for detection of its biodegradation in water.<br>**Results:** Results showed that the halflife (t1/2) of DBP biodegradation was 3.60 day when the concentration of DBP was 400 mg/L and the biomass concentration was 2 g/L. The biodegradation process conformed to the first-order kinetic model. Moreover, the whole degradation process could be divided into several steps: adsorption, desorption and degradation. Two metabolites of DBP degradation were identified as mono-butyl phthalate and phthalic acid by gas chromatography-mass spectrometry, which confirmed the dioxygenate process during the hydrolysis of DBP. | Wu et al, 2009 |
| **Type/Objective:** Numerous experiments have shown that the bioaccumulation of PAEs occurred in the aquatic and terrestrial food chain; meanwhile, it was found that some of PAEs were considered as potential carcinogens, teratogens and mutagens.<br>**Method:** In this research, two vertical/reverse-vertical flow constructed wetland systems were set up to study its removal efficiency of DBP pollution.<br>**Results:** The results showed that the constructed wetland system could remove DBP effectively, and the removal rates reached nearly 100%. Substrate microorganism and | Liang et al, 2009 |

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| enzymatic activities probably played key roles during DBP removal, and the removal of DBP probably mainly took place in the upper layer of chamber A in the constructed wetland systems. | |
| **Type/Objective:** DBP is commonly found in wastewater, sewage sludge, and aquatic environments. It has been classified as suspected endocrine disruptors by most countries.<br>**Method:** In this study, we isolated two DBP degradable strains from activated sludge. The strains were identified with their 16S rRNA as Deinococcus radiodurans and Pseudomonas stutzeri. We constructed the optimal condition of DBP degradation by using different kinds of incubation factors such as temperature, initial pH, yeast extract and surfactants.<br>**Results:** The optimal conditions of DBP degradation for these two strains are: 30 degrees C, pH 7.5 and static culture. Besides, addition of 0.23 mM of Triton X-100 could enhance the DBP degradation for D. radiodurans. In the end, we amended these two strains into the origin activated sludge and analyzed the whole microbial community structure of mixed cultures by PCR-DGGE technique. The result showed that only D. radiodurans could survive in the activated sludge after 7d of incubation. | Liao et al, 2010 |
| **Type/Objective:** This study investigated the effects of various culture treatments on di-n-butyl phthalate (DBP) degradation and the survival conditions of DBP-degrading bacterial strains in a soil microcosm.<br>**Method:** In the previous study, a DBP-degrading strain was isolated from activated sludge and identified by 16S rRNA as Deinococcus radiodurans. In this study, we added D. radiodurans into a soil microcosm and analyzed the structure of the whole bacterial community of the soil using a polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) technique. Meanwhile, the optimal conditions for DBP degradation were assessed by varying the temperature and initial pH of the culture, and by adding yeast extract and surfactants.<br>**Results:** The results show that the optimal conditions for DBP degradation in soil are a temperature of 35 degrees C, a pH of 7, and the addition of Triton X-100 and yeast extract. Furthermore, the addition of D. radiodurans can also enhance DBP degradation in soil. The PCR-DGGE analysis showed that D. radiodurans could survive in the soil microcosm through 24 days of incubation. | Liao, 2010 |
| **Type/Objective:** Degradation of DBP by bacteria in river sludge<br>**Results:** A gram negative isolate designated JDC-41 was obtained from river sludge using mixtures of phthalate esters as the sole source and energy. The isolate was identified as Ochrobactrum sp. based on its 16S rRNA gene sequence. Over 87% of supplied DBP was degraded by JDC-41 in a pH neutral mineral salts medium at 30 degrees C within 48 h. Increased DBP (50-500 mg/L) in the culture correspondingly increased degradation half-life from 3.83 to 18.12 h. DBP induced cells more rapidly degraded DBP. | Wu et al, 2010 |
| **Type/Objective:** Degradation of DBP in a landfill<br>**Conclusion:** DBP biodegradation was obviously accelerated with the operation of leachate recirculation compared to the conventional operation, and it was further promoted with the introduction of methanogenic reactor<br>**Method:** Considering the refuse and leachate as one whole system, a conventional landfill (CL) was set as a control, transformation of DBP in recirculated landfill (RL) and bioreactor landfill (BL) was studied.<br>**Results:** DBP was detected in both leachate and refuse from CL, RL and BL. The initial DBP amount was 18.5 microg x g(-1) in the landfill refuse. In addition, the stabilization process of landfill, with sequences of BL > RL > CL, played an important role on the biodegradation of DBP in refuse. Compared to the acidic | Fang et al, 2012 |

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| environment, the methanogenic environment was beneficial for DBP degradation. At the day of 310, refuse sedimentation rates were 7.0%, 11.9% and 24.3% in CL, RL and BL, respectively. DBP residual amounts were 2.1, 1.3 and 0.8 microg x g(-1), and its removal rates were 89.5%, 93.9% and 96.6% in the refuse from CL, RL and BL, respectively. The residual amounts of DBP with significant differences well fitted exponential decay models in CL, RL and BL. | |
| **Type/Objective:** Cylindrotheca closterium, a marine benthic diatom, was inoculated on the surface of marine sediments spiked with diethyl phthalate (DEP) and dibutyl phthalate (DBP) to investigate the effects of benthic microalgae on the degradation of the contaminants.<br>**Method:** The elimination of DEP and DBP from unsterilized sediments with C. closterium (treatment BA) was compared with that from unsterilized sediments without C. closterium (treatment B), sterilized sediments with C. closterium (treatment A) and sterilized sediments without C. closterium (treatment N).<br>**Results:** The results showed that during the 8-day experiment, inoculation with C. closterium increased the removal rates of the contaminants from the sediments, and more significantly from the surface layer (top 0.5 cm) of sediments than from the bottom layer of sediments. In the surface sediments, the first-order elimination rate constants (k) of DEP and DBP were in the order of treatment BA (2.098 and 0.309 d(-1))>treatment B (0.460 and 0.256 d(-1))>treatment A (0.216 and 0.039 d(-1))>treatment N (nil (no data)), indicating that microbial degradation played a major role in the removal of the contaminants from the sediments. A similar trend was also observed in bottom sediments (0.444 and 0.165 d(-1) in treatment BA, 0.329 and 0.194 d(-1) in treatment B, 0.129 d(-1) and nil in treatment A), but the difference of k values between treatments BA and B was relatively small. The positive effect of C. closterium on total phospholipid fatty acid (PLFA) content in sediments was observed, which was mainly related to the increase of biomass of aerobic bacteria as a result of improved sediment oxygenation and release of exudates (e.g. exopolysaccharides) by C. closterium. Moreover, Pearson correlation analysis showed a significant positive correlation between the elimination ratios of the contaminants and abundance of total aerobic bacterial PLFAs, suggesting that aerobic bacteria played a key role in C. closterium-promoted degradation of the contaminants in sediments. | LI, Gao, and Chi, 2015 |

## BACKGROUND INFORMATION FOR TABLE 42: BIOACCUMULATION

| Summary of Study | Reference |
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| **Type/Objective:** Metabolism studies were conducted to determine the uptake, degradation and residue composition of (pesticide, plasticizer) PAE (phthalic acid esters) residues in **fish**.<br>**Conclusion:** Residues and metabolism of PAEs in fish are described.<br>**Method:** Analytical methods were developed applicable to gas-liquid chromatography (GLC) determination of residues which may be associated with the presence of PAE in the aquatic environment. PAE and metabolite identities were also confirmed by GLC mass spectrometry and computer processing of spectral information. Channel catfish (Ictalurus punctatus) and fathead minnow (Pimephales promelas) from various locations in the USA were analyzed for DEHP (di-2-ethylhexyl phthalate) by GLC.<br>**Results:** Residue levels ranged from 0.2-10.0 mug/g on a whole **fish** basis. Higher PAE residues appear to be associated with industrial areas. Significant residues also occurred in commercially reared channel catfish and dietary contamination was suspected as the source of the phthalate ester. Static exposures of catfish (2g) to 1 mug/l of 14-C-labeled DEHP for 24 hr resulted in tissue residues of 2.6 mug/g. The composition of the radioactive residues was examined by TLC (thin layer chromatography). Four metabolic products were separated and identified as: mono-2 ethylhexyl phthalate, the corresponding monoester glucuronide (unknown aglycone), phthalic acid and a phthalic acid glucuronide. The monoester was the predominant metabolite identified by TLC. DBP (di-n-**butyl phthalate**) was metabolized in vitro 16x more rapidly than DHP by hepatic microsomes from male channel catfish. Formation of monoester metabolites of di-n-butyl and di-2-ethylhexyl phthalates was not identified by CO and did not require NADPH. Formation of 3 other unknown metabolites, however, was inhibited by CO and required NADPH. The phthalic acid monoesters were not further metabolized and appeared to be a terminal metabolite for this in vitro system. | Stalling et al, 1973 |

**Addendum to Table 42 – Bioaccumualtion Studies Not Useful for Classification**

| Summary of Study | Reference |
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| **Type/Objective:** Summary of data<br>**Conclusion:** The presence and effects of di-n-butylphthalate (DBP) in **aquatic** environments are reviewed. Particular attention is paid to the use of DBP as a solvent in the pesticide formulation, Aquagard, used to treat salmonids against external parasites at aquaculture sites in Europe. DBP is sparingly soluble in water, is readily metabolized by fish, and has a high lethal threshold for **aquatic** organisms. It is, however, lipophilic, persistent in **aquatic** sediment and is listed as a priority pollutant by the governments of Canada and United States. The dissemination of this compound directly to water raises several concerns. | Burridge and Haya, 1995 |
| **Type/Objective:** Environmental measurements of phthalates<br>**Conclusion:** Our data suggested that DEHP level in river sediments were influenced by water quality parameters due to their effects on the biodegradation processes, and that the DEHP level in fish was affected by fish habitat and physiochemical properties of polluted contaminants.<br>**Method:** Phthalate compounds in sediments and fishes were investigated in 17 Taiwan's rivers to determine the relationships between phthalate levels in sediment and **aquatic** factors, and biota-sediment accumulation factor (BSAF) for phthalates.<br>**Results:** Mean concentrations (range) of di(2-ethylhexyl) phthalate (DEHP), butyl benzyl phthalate (BBzP) and di-n-**butyl phthalate** (DBP) in sediment at low-flow season were 4.1 (<0.05-46.5), 0.22 (<0.05-3.1) and 0.14 (<0.05-1.3)mgkg(-1)dw; those at high-flow season were 1.2 (<0.05-13.1), 0.13 (<0.05-0.27) and 0.09 (<0.05-0.22)mgkg(-1)dw, respectively. Trace levels of dimethyl phthalate (DMP), diethyl phthalate (DEP) and di-n-octyl phthalate (DOP) in sediment were found in both seasons. Concentrations of DEHP in sediments were significantly affected by temperature, suspended solids, ammonia-nitrogen, and chemical oxygen demand. The highest concentration of DEHP in fish samples were found in Liza subviridis (253.9mgkg(-1)dw) and Oreochromis miloticus niloticus (129.5mgkg(-1)dw). BSAF of DEHP in L. subviridis (13.8-40.9) and O. miloticus niloticus (2.4-28.5) were higher than those in other fish species, indicating that the living habits of fish and physical-chemical properties of phthalates, like logKow, may influence the bioavailability of phthalates in fish. | Huang et al, 2008 |
| **Species:** Nine individual samples of 18 marine species were collected between June and September 1999. 3 samples of each species were collected from each of the 3 sampling station in the False Creek harbor a small (4x0.3 km) shallow (mean depth 8m) embayment of Burrard Inlet (Vancouver, BC). all samples were placed immediatly in aluminium foil and on ice in the field and then frozen at -20°C prior to analysis.<br><br>**Results:** Dialkyl phthalate esters concentrations (including DBP) were determined in 18 marine species representing approximately 4 trophic levels from their natural habitat (False Creek harbor, Vancouver, BC). Lipid equivalent concentrations in phthalates esters were determined and foodweb magnification factors were calculated and analysed regarding the trophic levels.<br><br>Food web characterization was determined on basis of 2 methods (trophic position model and stable nitrogen isotope analysis).<br><br>With biomagnification factors of 0.81 and 0.70 according to stable nitrogen isotope | REACH Dossier, 2015 |

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| and trophic position level, DBP do not biomagnify in the aquatic marine food web studied.<br><br>**Remarks:** Highest concentration in DBP occured in green macroalgea and plankton with 11.7 µg/g lipid.<br>The lipid equivalent concentration of the high molecular phthalate esters significaltly declined with increasing trophic position and stable nitrogen isotope in the food web ($p<0.05$).<br>DBP concentrations in the biota were within previously reported range concentration in British Columbia, Great Lakes region, United States and Northern Europe. | |
| Cai et al. (2008) studied the uptake of 5 polycyclic-aromatic hydrocarbons and 2 phthalic acid esters (including DBP) in radish (*Raphanus sativus*). Seeds were grown in latosolic red soil free of contamination and in soil ameneded with 1, 2 or 4 % of sewage sludge containing a mix of all theses organic contaminants.<br><br>Sludge contained 3.7 mg/kg dw of DBP. Plants were grown during 64 days before harvest.<br><br>Concentration of DBP in plant (shoot and roots separatly) and in substrates, have been measured after harvest with GC-MS method and BCF were deduced from these concentrations.<br><br>In roots or in shoots, and whatever the concentration in DBP in substrate, the BCF where < 1.<br><br>Therefore in the test conditions, DBP is not bioaccumulated by radish | (REACH, 2015). |

## BACKGROUND INFORMATION FOR TABLE 43: ACUTE AQUATIC HAZARD

| Summary of Study | Reference |
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| **Type/Objective:**<br>**Species/strain:** Algae - C. pyrenoidosa<br>**Method:** Fresh water. Measured DBP concentration. 96-hr duration. Inhibition of growth measured.<br>**Results:** EC50 >13 mg/L<br>**Conclusion:** | #33 (Yan et al, 1995) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Algae - S. subspicatus<br>**Method:** Fresh water. Nominal DBP concentration. 48-hr duration. inhibition of cell multiplication measured.<br>**Results:** EC50 = 3.5 mg/L<br>**Conclusion:** | #96 (Kuhn and Pattard, 1990) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Algae - S. subspicatus<br>**Method:** Fresh water. Nominal DBP concentration. 48-hr duration. Growth rate measured.<br>**Results:** EC50 = 9.0 mg/L<br>**Conclusion:** | #96 (Kuhn and Pattard, 1990) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Algae - S. subspicatus<br>**Method:** Fresh water. Measured DBP concentration. 72-hr duration. Cell growth measured.<br>**Results:** EC50 = 1.2 mg/L. NOEC (LOEC) = 0.5 mg/L<br>**Conclusion:** | #100 (Scholz, 1995) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Algae - S. subspicatus<br>**Method:** Fresh water. Measured DBP concentration. 72-hr duration. Growth rate measured.<br>**Results:** EC50 = 2.0 mg/L. NOEC (LOEC) = 0.5 mg/L<br>**Conclusion:** | #100 (Scholz, 1995) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Algae - G. breve<br>**Method:** Saltwater. Nominal DBP concentration. 96-hr duration. Growth rate measured.<br>**Results:** EC50 = 0.0034 – 0.2 mg/L<br>**Conclusion:** | #36 (Wilson et al, 1978) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Algae - G. breve<br>**Method:** Saltwater. Nominal DBP concentration. 96-hr duration. Cell number measured.<br>**Results:** EC50 = 0.02 – 0.6 mg/L | #36 (Wilson et al, 1978) cited in Staples et al, 1997 |

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| **Conclusion:** | |
| **Type/Objective:**<br>**Species/strain:** Midge Chironomus plumosus<br>**Method:** Freash water. Nominal DBP concentration. 48-hr duration ($3^{rd}$ – $4^{th}$ instar)<br>**Results:** LC50 = 0.76 mg/L<br>**Conclusion:** | #15 (Suggatt and Foote, 1981) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Midge C. plumosus<br>**Method:** Fresh water. Nominal DBP concentration. 48-hr duration ($3^{rd}$ – $4^{th}$ instar)<br>**Results:** LC50 = 5.46 mg/L<br>**Conclusion:** | #15 (Suggatt and Foote, 1981) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Midge C. plumosus<br>**Method:** Fresh water. Nominal DBP concentration. 48-hr duration ($2^{nd}$ instar)<br>**Results:** LC50 = 4.0 mg/L<br>**Conclusion:** | #15 (Suggatt and Foote, 1981) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Midge C. plumosus<br>**Method:** Fresh water. Nominal DBP concentration. 48-hr duration ($3^{rd}$ instar)<br>**Results:** LC50 = 5.4 mg/L<br>**Conclusion:** | #65 (Mayer and Ellersieck, 1986) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Measured DBP concentration. 48-hr duration.<br>**Results:** LC50 = 3.7 mg/L<br>**Conclusion:** | #39 (Call et al, 1983) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Measured DBP concentration. 48-hr duration static.<br>**Results:** LC50 = 3.0 mg/L<br>**Conclusion:** | #14 (Adams et al, 1995) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Nominal DBP concentration. 48-hr duration static renewal.<br>**Results:** LC50 = 5.2 mg/L<br>**Conclusion:** | #38 (McCarthy and Whitmore, 1985) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Measured DBP concentration. 24-hr duration. Immobilization measured.<br>**Results:** EC50 = 4.1 mg/L<br>**Conclusion:** | #40 (Scholz, 1994) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Measured DBP concentration. 48-hr duration. Immobilization measured.<br>**Results:** EC50 = 3.4 mg/L<br>**Conclusion:** | #40 (Scholz, 1994) cited in Staples et al, 1997 |

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| **Type/Objective:**<br>**Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Nominal DBP concentration. 24-hr duration.<br>**Results:** LC50 = 17 mg/L<br>**Conclusion:** | #41 (Kuhn et al, 1989) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Water flea Moina macrocopa<br>**Method:** Fresh water. Nominal DBP concentration. 3-hr duration.<br>**Results:** LC50 > 10 mg/L<br>**Conclusion:** | #26 (Yoshioka et al, 1985) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Scud Gammarus pseudolimnaeus<br>**Method:** Fresh water. Nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 = 2.10 mg/L<br>**Conclusion:** | #53 (Sanders et al, 1973) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Crayfish Orconectes nais<br>**Method:** Fresh water. Nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 > 10.0 mg/L<br>**Conclusion:** | #53 (Sanders et al, 1973) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Nematode larvae Panagrellus redivivus<br>**Method:** Fresh water. Nominal DBP concentration. 96-hr duration. Molting success measured.<br>**Results:** NOEC (LOEC) = 0.0028 mg/L<br>**Conclusion:** | #49 (Samoiloff et al, 1980) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Midge P. parthenogenica<br>**Method:** Fresh water. Measured DBP concentration. 48-hr static.<br>**Results:** LC50 = 6.29 mg/L<br>**Conclusion:** | #14 (Adams et al, 1995) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Midge P. parthenogenica<br>**Method:** Saltwater. Nominal DBP concentration. 24-hr duration.<br>**Results:** LC50 = 8.0 mg/L<br>**Conclusion:** | #101 (Hudson et al, 1981) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Mysid shrimp M. bahia<br>**Method:** Saltwater. Measured DBP concentration. 96-hr duration. Static.<br>**Results:** LC50 = 0.50 mg/L<br>**Conclusion:** | #14 (Adams et al, 1995) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Copepod N. spinipes<br>**Method:** Saltwater. Nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 = 1.7 mg/L<br>**Conclusion:** | #88 (Linden et al, 1979) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Channel catfish Ictalurus punctatus<br>**Method:** Nominal DBP concentration. 96-hr duration<br>**Results:** LC50 = 2.91 mg/L<br>**Conclusion:** | #54 (Mayer and Sanders, 1973) cited in Staples et al, 1997 |

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| **Type/Objective:**<br>**Species/strain:** Channel catfish Ictalurus punctatus<br>**Method:** Measured DBP concentration. 96-hr flow-through.<br>**Results:** LC50 = 0.46 mg/L<br>**Conclusion:** | #65 (Mayer and Ellersieck, 1986) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Bluegill L. macrochirus<br>**Method:** Measured DBP concentration. 96-hr duration. Static.<br>**Results:** LC50 = 0.48 mg/L<br>**Conclusion:** | #14 (Adams et al, 1995) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Bluegill L. macrochirus<br>**Method:** nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 = 0.73 mg/L<br>**Conclusion:** | #54 (Mayer and Sanders, 1973) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Bluegill L. macrochirus<br>**Method:** Nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 = 2.10 mg/L<br>**Conclusion:** | #65 (Mayer and Ellersieck, 1986) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Bluegill L. macrochirus<br>**Method:** Nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 = 1.58 mg/L<br>**Conclusion:** | #65 (Mayer and Ellersieck, 1986) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Bluegill L. macrochirus<br>**Method:** Nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 = 1.2 mg/L<br>**Conclusion:** | #63 (Buccafusco et al, 1981) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Bluegill L. macrochirus<br>**Method:** Nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 = 2.05 mg/L<br>**Conclusion:** | #65 (Mayer and Ellersieck, 1986) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Bluegill L. macrochirus<br>**Method:** Nominal DBP concentration. 96-hr duration. Flow-through.<br>**Results:** LC50 = 1.55 mg/L<br>**Conclusion:** | #65 (Mayer and Ellersieck, 1986) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Rainbow trout O. mykiss<br>**Method:** Measured DBP concentration. 96- hr duration.<br>**Results:** LC50 = 1.2 – 1.8 mg/L<br>**Conclusion:** | #66 (Hrudey et al, 1976) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Rainbow trout O. mykiss<br>**Method:** Nominal DBP concentration. 96-hr flow-through.<br>**Results:** LC50 > 1.24 mg/L<br>**Conclusion:** | #65 (Mayer and Ellersieck, 1986) cited in Staples et al, 1997 |

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| **Type/Objective:**<br>**Species/strain:** Rainbow trout O. mykiss<br>**Method:** Nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 = 2.56 mg/L<br>**Conclusion:** | #65 (Mayer and Ellersieck, 1986) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Rainbow trout O. mykiss<br>**Method:** Nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 = 6.47 mg/L<br>**Conclusion:** | #54 (Mayer and Sanders, 1973) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Rainbow trout O. mykiss<br>**Method:** Measured DBP concentration. 96-hr duration. flow-through.<br>**Results:** LC50 = 1.6 mg/L<br>**Conclusion:** | #14 (Adams et al, 1995) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Rainbow trout O. mykiss<br>**Method:** Nominal DBP concentration. 96-hr duration. Flow-through.<br>**Results:** LC50 = 1.48 mg/L<br>**Conclusion:** | #65 (Mayer and Ellersieck, 1986) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Yellow perch Perca flavescens<br>**Method:** Measured DBP concentration. 96-hr duration. Flow-through.<br>**Results:** LC50 = 0.35 mg/L<br>**Conclusion:** | #65 (Mayer and Ellersieck, 1986) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Nominal DBP concentration. 48-hr duration.<br>**Results:** LC50 = 1.49 mg/L<br>**Conclusion:** | #54 (Mayer and Sanders, 1973) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 = 2.02 mg/L<br>**Conclusion:** | #38 (McCarthy and Whitmore, 1985) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Measured DBP concentration. 96-hr duration. Static.<br>**Results:** LC50 = 1.54 mg/L<br>**Conclusion:** | #14 (Adams et al, 1995) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Nominal DBP concentration. 96-hr duration.<br>**Results:** LC50 = 1.30 mg/L<br>**Conclusion:** | #54 (Mayer and Sanders, 1973) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Nominal DBP concentration. 96-hr duration. Flow-through.<br>**Results:** LC50 = 3.95 mg/L<br>**Conclusion:** | #65 (Mayer and Ellersieck, 1986) cited in Staples et al, 1997 |

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| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Measured DBP concentration. 96-hr duration. Flow-through.<br>**Results:** LC50 = 0.85 mg/L<br>**Conclusion:** | #52 (DeFoe et al, 1990) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Measured DBP concentration. 96-hr duration. Flow-through.<br>**Results:** LC50 = 1.1 mg/L<br>**Conclusion:** | #52 (DeFoe et al, 1990) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Measured DBP concentration. 96-hr duration. Flow-through.<br>**Results:** LC50 = 0.92 mg/L<br>**Conclusion:** | #14 (Adams et al, 1995) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Measured DBP concentration. 120-hr duration. Flow-through.<br>**Results:** LC50 = 0.92 mg/L<br>**Conclusion:** | #68 (Springborn Bionomics, 1983) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Measured DBP concentration. 144-hr duration. Flow-through.<br>**Results:** LC50 = 0.92 mg/L<br>**Conclusion:** | #68 (Springborn Bionomics, 1983) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Measured DBP concentration. 96-hr duration. Flow-through.<br>**Results:** LC50 = 0.61 mg/L<br>**Conclusion:** | #52 (DeFoe et al, 1990) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Fathead minnow P. promelas<br>**Method:** Measured DBP concentration. 96-hr duration. Flow-through.<br>**Results:** LC50 = 0.90 mg/L<br>**Conclusion:** | #52 (DeFoe et al, 1990) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Red killfish Oryzias latipes<br>**Method:** Measured DBP concentration. 96-hr duration. Static renewal.<br>**Results:** LC50 = 4.3 mg/L<br>**Conclusion:** | #26 (Yoshioka et al, 1985) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Zebrafish Brachydanio rerio<br>**Method:** Measured DBP concentration. 96-hr duration. Static renewal.<br>**Results:** LC50 = 2.2 mg/L<br>**Conclusion:** | #69 (Scholz, 1994) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Sheepshead minnow C. variegatus<br>**Method:** measured DBP concentration. 96-hr duration. Flow-through.<br>**Results:** LC50 >0.60 mg/L<br>**Conclusion:** | #14 (Adams et al, 1995) cited in Staples et al, 1997 |

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| **Type/Objective:** Acute toxicity of DBP and DEHP<br>**Conclusion:** It shows that the 96 h-LC50 is 16.30 mg/L for DBP.<br>**Method:** Carp were exposed to six different concentrations of DBP for 96 h.<br>**Results:** LC50 = 16,30 mg/L**.** | Zhao et al, 2014 |

**Addendum to Table 43 – Acute Aquatic Studies Not Useful for Classification**

| Summary of Study | Reference |
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| **Type/Objective:**<br>**Species/strain:**<br>**Method:**<br>**Results:** DBP inhibits growth and photosynthesis of green algae (*Chlorella emersonii* CCAP strain 211/8 h and *Selenastrum capricornutum* CCAP strain 278/4) at concentrations higher than 10-$^{5}$*M*. The $IC_{50}$ value for $CO_2$-dependent oxygen evolution in algae was $3 \times 10^{-4}$M. The $CO_2$-reduction in isolated protoplasts prepared from barley (*Hordeum vulgare* L. cv. Simba) was also inhibited by phthalate. The $IC_{50}$ value was $2 \times 10^{-4}$*M*. The electron transport in isolated thylakoids prepared from spinach was inhibited with an $IC_{50}$ value of $3 \times 10^{-4}$*M*. The $IC_{50}$ value for uncoupled electron transport extrapolated to zero chlorophyll concentration was $2.5 \times 10^{-5}$*M*. The effect of DBP was localized to reactions in photosystem II. DBP could thus be a pollutant which affects growth and photosynthesis of plants.<br>**Conclusion:** | Melin and Egneus, 1983 |
| **Type/Objective:** Frog Embryo Teratogenesis Assay<br>**Conclusion:** The pooled 96-hr LC50 and EC50, as well as the MCIG were 5 ppm DBP. Analogous to the findings in rodents and rabbits, DBP unequivocally alters the normal development of Xenopus laevis tadpoles.<br>**Method:** Embryos were injected with human chorionic gonadotropin and exposed to 0, 1, 5, 7.5, or 10 ppm DBP in a static-24 hr-renewal-system from 6 to 96 hr post-fertilization. The assay was terminated at 96 hr when primary organogenesis is normally complete. Mortality, developmental malformations and body length along with developmental stage of surviving tadpoles were monitored to determine the LC50, EC50, and minimum concentration to inhibit growth (MCIG).<br>**Results:** The percent mortality rates for 0, 1, 5, 7.5, and 10 ppm DBP were 3, 3, 52, 96 and 100%, respectively. The incidences of developmental malformations for 0 and 1 ppm DBP were 4 and 8%, while for 5 and 7.5 ppm DBP they reached 50 and 100%. Of the developmentally malformed tadpoles in the 5 ppm group, 13% had axial alterations, 88% had cardiac alterations, 42% had gut alterations, and 38% had optic alterations; the corresponding defects in the 7.5 ppm group were 0%, 100%, 50%, and 50%. Mean body length and progression of normal development were significantly affected at 5 and 7.5 ppm. | Higuchi et al, 2000 |
| **Type/Objective:** Some chemicals might act as aneugens, substances that cause numerical chromosomal aberrations (NCAs).<br>**Conclusion:** DBP induced numerical chromosomal aberrations and impaired larval fitness in a marine worm at concentrations of 1 to 5 µM.<br>**Method:** Fluorescence in situ hybridisation technique (FISH) was used to detect NCAs in the interphase cell nuclei of Pomatoceros lamarckii, a tube-building annelid worm (invertebrate).<br>**Results:** When exposed either under acute or chronic (viz. adult) exposure conditions, colchicine and DBP, two recognised aneugens, induced significant increases in the levels of NCAs, in the dose range 1 to 5 µM, in both four to eight cell | Wilson et al, 2002 |

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| embryo stages and 24 h-old larvae. An inverse correlation was observed between the induced levels of NCAs and larval fitness based on the results of a standard 48-h larval bioassay. | |
| **Type/Objective:** Toxicity to embryogenesis and larval development in a marine bivalve<br>**Conclusion:** With the completion of metamorphosis as an endpoint, the 96-h NOEC of DBPwas 0.022 mg/L.<br>**Method:** The toxicity of seven phthalate esters, including DBP, to embryogenesis and larval development of the marine univalve Haliotis diversicolor supertexta was examined by means of two-stage embryo toxicity test.<br>**Results:** At the blastula stage, the normal embryonic development of H. diversicolor supertexta showed a good dose-response decrease when exposed to DMP, DEP, DBP, BBP, and DnHP. 9-h EC(50) values of DMP, DEP, DBP, BBP, and DnHP were 55.71, 39.13, 8.37, 2.65, and 3.32 mg/l, respectively. 9-h EC(50) values of DEHP and DOP were not available due to their low solubility. The toxicity order of seven tested PAEs was BBP>DnHP>DBP>DEP>DMP><br>DOP>DEHP. With the completion of metamorphosis as an experimental endpoint, the 96-h no-observed effect concentration values of DBP, DEHP and the other five tested PAEs were 0.022, 0.021, and 0.020 mg/l, respectively. | Liu Y et al, 2009 |
| **Type/Objective:** The canonical Wnt/β-catenin signaling pathway is critical during early teleost development for establishing the dorsal-ventral axis. Within this pathway, GSK-3β, a key regulatory kinase in the Wnt pathway, regulates β-catenin degradation and thus the ability of β-catenin to enter nuclei, where it can activate expression of genes that have been linked to the specification of the dorsal-ventral axis.<br>**Conclusion:** DBP and other compounds induced an increase in the levels of nuclear β-catenin throughout the embryo, indicating that the morphological abnormalities were a result of disruption of Wnt/β-catenin signaling during dorsal-ventral axis specification.<br>**Method:** Zebrafish embryos were exposed to commercially available GSK-3 inhibitors (GSK-3 Inhibitor IX and 1-azakenpaullone), or common environmental contaminants (DBP or the polycyclic aromatic hydrocarbons phenanthrene and fluorene) from the 2 to 8-cell stage through the mid-blastula transition (MBT).<br>**Results:** In this study, we describe the morphological abnormalities that resulted in zebrafish embryos when axis determination was disrupted by environmental contaminants. These abnormalities were linked to abnormal nuclear accumulation of β-catenin. Furthermore, we demonstrated that the developmental abnormalities and altered nuclear β-catenin accumulation occurred when embryos were exposed to commercial GSK-3β inhibitors.<br>These embryos displayed morphological abnormalities at 12.5 h post-fertilization (hpf) that were comparable to embryos exposed to lithium chloride (LiCl) (300 mM LiCl for 10 min, prior to the MBT), a classic disruptor of embryonic axis determination. Whole-mount immunolabeling and laser scanning confocal microscopy were used to localize β-catenin. The commercial GSK-3 Inhibitors as well as LiCl, DBP, fluorene and phenanthrene all induced an increase in the levels of nuclear β-catenin throughout the embryo, indicating that the morphological abnormalities were a result of disruption of Wnt/β-catenin signaling during dorsal-ventral axis specification. The ability of environmental chemicals to directly or indirectly target GSK-3β was assessed. Using Western blot analysis, the ability of these chemicals to affect enzymatic inhibitory phosphorylation at serine 9 on GSK-3β was examined, but no change in the serine phosphorylation state of GSK-3β was detected in exposed | Fairbaim et al, 2013 |

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| embryos. Furthermore, polycyclic aromatic hydrocarbons and DBP had no direct effect on the in vitro kinase activity of GSK-3β. While developmental abnormalities resulting from these axis-disrupting contaminants were linked to β-catenin accumulation in nuclei. | |
| **Type/Objective:** Zebrafish embryos were used to assess the neurotoxicity of DBP, diethyl phthalate (DEP) and their mixture.<br>**Conclusion:** These results indicate that DBP and DEP have the potential neurotoxicity in zebrafish embryos.<br>**Method:** Four-hour post-fertilization (hpf) zebrafish embryos were exposed to various concentrations of DBP, DEP and their mixture (DBP-DEP) until 96 hpf. The transcriptions levels of selected neuron-related genes reported as neurotoxicity biomarkers were analyzed.<br>**Results:** Transcripts of growth associated protein 43 (gap43), embryonic lethal abnormal vision-like 3 (elavl3), glial fibrillary acidic protein (gfap), myelin basic protein (mbp), α1-tubulin and neurogenin1 (ngn1) were significantly up-regulated after DBP, DEP and DBP-DEP mixture exposure. In addition, acetylcholinesterase activity was significantly inhibited in the embryos. | Xu et al, 2013a |
| **Type/Objective:** Antioxidant and immune responses in zebrafish embryos were assessed in zebrafish embryos exposed to DBP and DEP, separately and together.<br>**Conclusion:** The results demonstrated that DBP/DEP exposure could induce the antioxidant and immune responses in zebrafish embryos.<br>**Method:** we analyzed the oxidative stress related indices and immune related gene expression of zebrafish embryos after a short-term exposure to various concentrations of di-n-butyl phthalate (DBP), diethyl phthalate (DEP) and their mixture (DBP-DEP) from 4 h post-fertilization (hpf) to 96 hpf.<br>**Results:** Exposure to the chemicals was found to enhance the production of reactive oxygen species (ROS) and lipid peroxidation (LPO) in a concentration-dependent manner. Simultaneously, adaptive responses to DBP/DEP-induced oxidative stress were observed. The activity of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were all increased in a concentration-dependent manner. The transcription of innate immune related genes including interferon γ (IFNγ), interleukin-1β (IL1β), Myxovirus resistance (Mx), tumor necrosis factor α (TNFα), CC-chemokine, CXCL-clc, lysozyme (Lyz) and complement factor C3B (C3) were up-regulated upon DBP, DEP and their mixture exposure, suggesting the induction of immune response. In addition, co-exposure to DBP-DEP also induced antioxidant defense and immune response in zebrafish embryo. | Xu H et al, 2013b |
| **Type/Objective: Toxicity to aquatic plants**<br>**Method: Aquatic** toxicity data of 126 priority pollutants were screened and analyzed in this study. Through data analysis, five priority pollutants namely 1,1,1-trichloroethane (1,1,1-TCA), 4-nitrophenol (4-NP), butylbenzyl phthalate (BBP), di-n-**butyl phthalate** (DBP) and N-nitrosodimethylamine (NDMA) were identified to have high phytotoxicity effect.<br>**Results:** The most sensitive **aquatic** plants to these five pollutants are all alage, including Chlamydomonas reinhardtii, Pseudokirchneriella subcapitata, Gymnodinium breve. The water quality criteria concentration of the five pollutants were derived by the species sensitivity distribution method. The acute criteria concentration for the five pollutants were derived to be 1474, 2180, 54.41, 98.52 and 520.4 μg L(-1), and the chronic criteria concentration for them were 147.4, 218.0, 5.441, 9.852 and 52.04 μg L(-1), respectively. | Yan et al, 2015 |
| **Type/Objective:** This study set out to understand the immune-toxic effects of dibutyl | Xu et al, 2015 |

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| phthalate (DBP) using transgenic, albino or AB line zebrafish.<br>**Conclusion:** The overall results indicate that DBP in aquatic environment greatly influence the immune system in fish.<br>**Method:** Zebrafish embryos were exposed to different concentrations of DBP, and the immune cells formation, phagocytosis ability were measured after a short-term exposure to DBP for 6 h post-fertilization (hpf) to 72 or 96 hpf.<br>**Results:** Exposure to DBP was found to inhibit the neutrophils and macrophage formation in a concentration-dependent manner. The ability of macrophage phagocytosis was all decreased after exposure to DBP, indicating the occurrence of immunotoxicity. The respiratory burst was induced, and the transcription levels of T/B cell-related genes rag1/2 were up-regulated. | |
| **Type/Objective:** The inhibitory action and possible damage mechanism of dibutyl phthalate (DBP) on the red tide algae Karenia brevis were investigated.<br>**Conclusion:** The results showed that the algae experienced oxidative stress after exposure to 5 mg/L DBP and that mitochondria could be the main target sites for DBP attack.<br>**Results:** The results showed that the algae experienced oxidative stress after exposure to 5mgL(-1) DBP. Malondialdehyde (MDA) peaked after 72h, with a value approximately 2.3 times higher than that observed for untreated cells. The superoxide dismutase (SOD) and catalase (CAT) activities significantly increased as an adaptive reaction after 48h. DBP induced the overproduction of reactive oxygen species (ROS), the OH concentration showed a peak of 33UmL(-1) at 48h, and the highest H2O2 content was approximately 250nmol/10(7) cells at 72h; these latter two values were 2.5 and 4.4 times higher than observed for the control, respectively. TEM images showed that a number of small vacuoles or apical tubers were commonly found around the cell membrane, and the membrane structure was ultimately disintegrated. Further experiments were carried out to locate the original ROS production sites following DBP exposure. The activity of CuZn-SOD (a mainly cytosolic isoform, with some also found in chloroplasts) under DBP exposure was approximately 2.5 times higher than the control, whereas the Mn-SOD (mitochondrial isoform) activity was significantly inhibited. No significant difference was observed in the activity of Fe-SOD (chloroplastic isoform). In addition, dicumarol (an inhibitor of the electron transport chain in the plasma membrane) stimulated DBP-induced ROS production, whereas rotenone (an inhibitor of the mitochondria electron transport chain complex I) decreased DBP-induced ROS production. | Li et al, 2015 |

## BACKGROUND INFORMATION FOR TABLE 44: CHRONIC AQUATIC TOXICITY

| Summary of Study | Reference |
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| **Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Measured DBP concentration. 21-d duration. Survival measured.<br>**Results:** NOEC (LOEC) = 0.96 mg/L | #50 cited in Staples et al, 1997 |
| **Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Measured DBP concentration. 21-d duration. Survival/reproduction measured.<br>**Results:** EC50 = 0.20 mg/L NOEC (LOEC) = 0.11 mg/L | #52 (DeFoe et al, 1990) cited in Staples et al, 1997 |
| **Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Measured DBP concentration. 21-d duration. Survival/reproduction measured.<br>**Results:** EC50 = 1.92 mg/L NOEC (LOEC) = 1.05 mg/L | #52 (DeFoe et al, 1990) cited in Staples et al, 1997 |
| **Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Measured DBP concentration. 21-d duration. Survival/reproduction measured.<br>**Results:** EC50 = 0.46 mg/L NOEC (LOEC) = 0.16 mg/L | #52 (DeFoe et al, 1990) cited in Staples et al, 1997 |
| **Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Measured DBP concentration. 21-d duration. Survival/reproduction measured.<br>**Results:** MATC = 0.32 mg/L | #41 (Kuhn et al, 1989) cited in Staples et al, 1997 |
| **Species/strain:** Water flea D. magna<br>**Method:** Fresh water. Nominal DBP concentration. 16-d duration. Fecundicity measured.<br>**Results:** NOEC (LOEC) = 0.56 mg/L | #38 (McCarthy and Whitmore, 1985) cited in Staples et al, 1997 |
| **Type/Objective:** Hatchability and growth of rainbow trout, *Oncorhynchus mykiss*<br>**Method:** egg hatchability and survival, fry survival and growth measured as length and weight<br>**Results:** According to a review of long-term toxicity tests in fish (IPCS/WHO, 1997), the lowest NOEC among available studies was observed in a 99-day test (60 days posthatch) reported by Ward and Boeri (1991). The 99-day NOEC (growth) was 100 µg/litre (60 days posthatch), a 99-day LOEC was 190 µg/litre (growth reduced by about 27%) and 100% mortality occurred by day 40 at 400 µg/litre. | Ward and Boeri, 1991 as cited in IPCS/WHO, 1997 |
| **Type/Objective:**<br>**Species/strain:** Algae - Chlorella emersoni<br>**Method:** Fresh water. Nominal DBP concentration. 7 d duration. Growth and photosynthesis assessed.<br>**Results:** NOEC (LOEC) = 2.78 mg/L<br>**Conclusion:** | #32 (Melin and Egneus, 1983) cited in Staples et al, 1997 |

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| **Type/Objective:**<br>**Species/strain:** Algae - S. capricornutum<br>**Method:** Fresh water. Measured DBP concentration. 10-d duration. Static conditions. Cell number measured.<br>**Results:** NOEC (LOEC) = 0.21 mg/L<br>**Conclusion:** | #28 (Springobrn Bionomics, 1984) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Algae - S. capricornutum<br>**Method:** Fresh water. Nominal DBP concentration. 7-d duration. Growth and photosynthesis measured.<br>**Results:** NOEC (LOEC) = 2.78 mg/L<br>**Conclusion:** | #32 (Melin and Egneus, 1983) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Rainbow trout O. mykiss<br>**Method:** Measured DBP concentration. Growth and survival during 60-days after hatching.<br>**Results:** NOEC (LOEC) = 0.1 mg/L (MATC = 0.14 mg/L)<br>**Conclusion:** | #51 (Rhodes et al, 1995) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Algae - S. subspicatus<br>**Method:** Fresh water. Measured DBP concentration. 7-d duration. Growth rate measured.<br>**Results:** NOEC (LOEC) = 6.1 mg/L<br>**Conclusion:** | #99 (Huels, 1991) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Algae - Dunaliella parva<br>**Method:** Saltwater. Nominal DBP concentration. 7-d duration. Cell aggregation measured.<br>**Results:** NOEC (LOEC) = 0.28 mg/L<br>**Conclusion:** | #35 (Acey wt al, 1987) cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Amphipod Gammarus pulex<br>**Method:** Fresh water. Nominal DBP concentration. 25-d duration. Locomotor activity measured.<br>**Results:** NOEC (LOEC) = 0.10 mg/L<br>**Conclusion:** | #57 cited in Staples et al, 1997 |
| **Type/Objective:**<br>**Species/strain:** Grass shrimp P. pugio<br>**Method:** Saltwater. Measured DBP concentration. 30-d duration. Larval mortality measured.<br>**Results:** NOEC (LOEC) = 10.0 mg/L<br>**Conclusion:** | #62 (Laughlin et al, 1978) cited in Staples et al, 1997 |
| **Type/Objective:** To evaluate whether long-term exposures to environmentally relevant concentrations of DnBP disrupt the reproduction-based endpoints in juvenile Murray rainbowfish (Melanotaenia fluviatilis).<br>**Method:** Sexually undifferentiated Murray rainbowfish (30 days post hatch – approximately 10mg) were exposed in 1 litre of water in glass beakers to 5, 15 and 50 µg /L DnBP in a semi-static system for 30, 60 and 90 days. Water control and 0.0005% solvent control beakers were also used (solvent was same across all concentrations and control). Four beakers containing four fish in each were used (16 fish per treatment per time interval with 240 total fish). After 30 days of | Bhatia et al, 2014b |

exposure, the fish were transferred to beakers containing 2 litre of water to account for their growth. Oxygenation with very light bubbling was set up using capillaries attached to the aerators. The temperature was maintained at 23 °C and recorded every 5 seconds. The physical conditions (temperature and light intensity in the laboratory; and DO, pH and conductivity of water) during the test were similar to those during the acclimation period. Fish were fed 4% (w/w) baby brine shrimp once daily. The water in the testing beakers was renewed and spiked with fresh DnBP solutions daily.

The effects on survival, body growth, whole-body concentrations of sex steroid hormones and gonadal development were investigated. Histological examination was as follows:

The germ cells in the ovaries were identified on the basis of their size and the presence of cortical alveoli or yolk vesicles as outlined by Bhatia et al. (2013) and classified as follows:

(a) Perinucleolar oocytes had a large nucleus and homogenously staining, dark ooplasm. Cortical alveoli and yolk vesicles were absent;

(b) Cortical alveolar oocytes were larger than perinucleolar oocytes. The cortical alveoli were arranged in a ring near the periphery of the oocytes;

(c) Early vitellogenic oocytes showed a beginning of the appearance of yolk vesicles in the centre. The cytoplasm was filled with cortical alveoli; and

(d) Late vitellogenic oocytes had their entire cytoplasm filled with yolk vesicles and the cortical alveoli were pushed to the periphery.

The developmental stage of the ovaries was classified as follows:

(a) Stage 0 (immature): Only perinucleolar oocytes were present in this stage;

(b) Stage I (previtellogenic): Abundant perinucleolar oocytes and a few cortical alveolar oocytes were present;

(c) Stage II (vitellogenic): early vitellogenic oocytes can be seen in this stage. In addition, some perinucleolar and cortical alveolar oocytes are also present; and

(d) Stage III (mature): Abundant late vitellogenic oocytes with accumulated vitellogenic granules were present.

The testicular germ cells were classified according to the method of Bhatia et al. (2014a, b, c) as follows:

(a) Spermatogonia were large cells with eosinophilic cytoplasm arranged in groups of three to four near but not limited to the periphery of the testes;

(b) Spermatocytes had moderate amount of dark staining cytoplasm. These were arranged in clusters called the spermatocysts throughout the length of the testes;

(c) Spermatids were small cells with dense cytoplasm and were found in between the spermatocysts; and

(d) Spermatozoa were mature germ cells scattered in the tubular lumen. Thesewere the smallest in sizewithminimal cytoplasm.

The development of the testes was classified into the following stages:

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| (a) Stage 0 (immature): The testes consisted of spermatogonia and spermtaocytes only. No spermatozoa were present in this stage;<br>(b) Stage I (early-spermatogenic): spermatozoa begin to appear in this stage. Abundant spermatocytes were present;<br>(c) Stage II (mid-spermatogenic): Approximately similar proportions of spermatocytes, spermatids and spermatozoa were present; and<br>(d) Stage III (mature): All types of germ cells were present. However, the proportion of spermatozoa was the highest.<br>The concentrations of sex steroid hormones, E2 and 11-keto testosterone (11-KT) were measured in the whole-body homogenates using enzyme immunoassay (EIA) kits (as described in Bhatia et al. (2014)). Mortaltiy and vital indices were also recorded.<br><br>**Results:** The lowest observed effective concentration to affect the condition factor after 90 days was 5 µg/L . Complete feminization of the gonad was noted in fish exposed to 5 µg l(-1) for 90 days and to 15 and 50 µg /L of DnBP for 30 or 60 days. After 90 days of exposure to DnBP, the ovaries were regressed and immature as opposed to the control fish which were in early-vitellogenic stage. Testes, present only in fish exposed to 5 µg/L of DnBP for 30 or 60 days, were immature in comparison to the control fish that contained testes in the mid-spermatogenic phase. The E2/11-KT ratio was significantly higher only after exposures to 5 µg/L DnBP for 90 days and 50 µg/L DnBP for 30 days.<br><br>**Conclusion:** The data suggest that exposures to 5 µg/L DnBP for 30 days did not have profound effects on body growth and gonadal differentiation of fish. However, 30 days of exposure to 15 µg/L could interfere with the gonad development and to 50 µg/ could compromise the hormonal profile of juvenile fish. | |

**Addendum to Table 44 – Chronic Aquatic Toxicity Studies Not use for Classification**

| Summary of Study | References |
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| **Type/Objective:** To determine if DBP also affects species other than rat and if species differences exist, we studied the effects of DBP in an amphibian and a non-rodent mammal.<br>**Conclusion:** DBP increased mortality and delayed metamorphosis in Xenopus tadpoles exposed to 10 ppm<br>**Method:** Xenopus laevis tadpoles were exposed to 0 (n = 14) or 10 (n = 52) ppm DBP in a static alternate-day-water-renewal system beginning from 2 wk of life (stage 52). Mortality and time to complete metamorphosis (stage 66) were monitored weekly.<br>**Results:** The mortality rates in treated and control groups were 85% and 0% by wk 1 post-exposure, and 92% and 28% by wk 16. Whereas 75% of controls metamorphosed by wk 12 and 100% by wk 14, none of the treated ones completed metamorphosis until wk 16. Two groups (n = 6) of pregnant Dutch-Belted rabbits were treated with DBP or vehicle alone. DBP was administered orally in corn syrup at 400 ppm/kg body wt between gestation days 15 and 30. Development of male pups was monitored. At | Higuchi et al, 1999 |

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| 12 wk, body weights and the weights of testes and epididymides did not differ (p greater than .1) between groups, but the accessory gland weight and anogenital distance were lower (p less than .01) in treated pups. Analogous to that found in the rat, one treated rabbit had undescended testes, ambiguous genitalia, hypospadias, and missing prostate and bulbourethral glands. In addition to disrupting androgen-dependent events, DBP or its metabolite(s) may also disrupt thyroid hormone cascade since metamorphosis, a thyroid hormone-dependent event, was perturbed. | |
| **Type/Objective:** Japanese medaka, a freshwater teleost, was used to examine multigeneration reproductive effects of DBP.<br>**Conclusion:** DBP caused reproductive toxicity in the second generation medaka offspring, following chronic dietary exposure to environmentally relevant concentrations.<br>**Method:** The endpoints included mortality, histopathologic changes, growth, gonadal-somatic index (GSI), sexual development, fecundity, embryonal development, vitellogenin induction and hepatic microsomal testosterone metabolism. The F0 generation was first exposed as 14-day old larval **fish**. Exposure consisted of feeding DBP or 17 beta-estradiol (E2) in dry flake food, at a daily ration 5% body weight. There were seven treatment groups, with two replicates (n = 20). The treatment groups included an ethanol control, 0.5, 5, 50 ug DBP-g flake food, and 0.05, 0.5, 5 ug E2/g flake food. The F0 and F1 generation were fed each treatment through sexual maturation.<br>**Results:** All E2 0.05 ug/g **fish** were phenotypic females, as confirmed histologically. No eggs were produced by E2-treated **fish**. DBP had no major effects in the F0 generation. DBP at 50 ug/g decreased F1 generation ovary weight, GSI, egg production, and in the F1 generation males reduced testes weight and GSI. DBP at 50 ug/g caused an increase in microsomal protein levels, liver weight, and hepatic-somatic index in both F1 generation males and females. Evaluation of F1 and F2 eggs from DBP-treated groups, showed normal embryonic development. The male to female ratios in all DBP groups were similar to the control. | Patyna et al, 1999 (Abstract from meeting of Society of Toxicology) |
| **Type/Objective:** Metamorphosis and subsequent development of Xenopus laevis embryos<br>**Conclusion:** Chronic developmental exposure to DBP causes substantial mortality in Xenopus laevis at a relatively low concentration (1 ppm) and impairs metamorphosis at even lower levels.<br>**Method:** Xenopus embryos (n = 400/dose group) were exposed to 0.1, 0.5, 1, 5, 10, 15 ppm DBP in 0.01% DMSO, or vehicle alone (control) from 6 h post-fertilization (Nieuwkoop and Faber stage 8) until completion of metamorphosis at 12 wk (stage 66; when at least 90% of controls metamorphosed). During the initial 96 h of life, embryos were raised and evaluated according to FETAX procedures. Thereafter, tadpoles were raised in a static alternate-day-water-renewal system. Stage of development, metamorphic index (hindlimb:tail length ratio), and time to complete metamorphosis were determined in a random subpopulation (at least 25% of surviving tadpoles).<br>**Results:** During the first 96 h, mortality rates for 0, 0.1, 0.5, 1, 5, 10, and 15 ppm DBP were 5, 3, 5, 5, 7, 33, and 75%, respectively. At 96 h, the corresponding incidences of developmental malformations were 6, 8, 9, 3, 19, 39, and 91%. Progression of normal development and mean body length were significantly retarded (p less than 0.05) in the 10 (stage 45, 8.5 mm) and 15 ppm (stage 45, 8.4 mm) dose groups vs. controls (stage 46, 9.4 mm). Mortality reached 100% in 15 ppm group at 1 wk. At this age, mortality rates for 0, 0.1, 0.5, 1, 5, and 10 ppm DBP were 2, 2, 1, 40, 24, and 29%; an additional 18, 10, 16, 13, 41, and 95% died in these groups between 1 and 12 wk. Metamorphic index was significantly decreased (p less than 0.01) in all | Higuchi et al, 2001 |

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| DBP-treated groups at 12 wk. Reflective of this, only 76 (61/80), 80 (64/80), 68 (27/40), 35 (14/40) and 0% (0/8) of 0.1, 0.5, 1, 5 and 10 ppm groups completed metamorphosis at 12 wk vs. 96% (77/80) of controls. In those that completed metamorphosis after 12 wk, the delay ranged from 1 to 8 wk. Even at low-range dose levels (viz., 0.1 and 0.5 ppm) where there was no treatment-related mortality, metamorphosis was significantly delayed (p less than 0.01). | |
| **Type/Objective:** To investigate if xenoestrogens can cause proliferation of liver peroxisomes using zebrafish (Danio rerio) as a model.<br>**Conclusion:** All five tested compounds caused significant proliferation of liver peroxisomes ($p < 0.05$) as indicated by increased peroxisomal surface and numerical densities and elevated activities of the peroxisomal beta-oxidation enzyme acyl-CoA oxidase (AOX).<br>**Method:** Adult male zebrafish were exposed for 15 days to 17beta-estradiol (E2) and the xenoestrogens dibutylphthalate (DBP), methoxychlor (MXC), 4-tert-octylphenol (OP) and 17alpha-ethynylestradiol (EE2).<br>**Results:** In the case of DBP, MXC and E2, positive significant correlations between peroxisomal density parameters and AOX were found. The treatments did not produce gross alterations in testis histology, but spermatogenic cell proliferation was disturbed in E2 and EE2-treated groups and vitellogenin levels increased significantly in fish exposed to MXC, OP, EE2 and E2 with respect to controls. Furthermore, a significant correlation between vitellogenin levels and AOX activity was found for MXC, OP and EE2 treatments, suggesting that for the latter xenoestrogens early estrogenic effects are associated with liver peroxisome proliferation. No such association occurred with typical peroxisome proliferators such as DBP. | Ortiz-Zarragoitia and Cajaraville, 2005 |
| **Type/Objective:** Authors evaluated the effects of low concentrations of DBP on spermatogenesis in Xenopus laevis, the African clawed frog.<br>**Conclusion:** Subchronic exposure to low concentrations of DBP impairs spermatogenesis in Xenopus laevis frogs.<br>**Method:** Xenopus tadpoles were exposed to 0, 0.1, 0.5, 1.0, 5.0, or 10.0 ppm DBP, beginning at sexual differentiation (Nieuwkoop and Faber stage 52; 3 weeks of age) and continuing until 100% of controls metamorphosed (stage 66; 8 weeks of age).<br>**Results:** Upon necropsy at 33 weeks, 4-6% of DBP-treated frogs had only one testis, and 2-4% had retained oviducts. In all DBP treatment groups, seminiferous tubule diameter and the average number of germ cell nests per tubule were lower, and the number of tubules with no germ cells was significantly higher ($p < 0.05$). The percent of secondary spermatogonial cell nests significantly decreased ($p < 0.05$) in 1.0, 5.0, and 10.0 ppm groups. Several lesions occurred in DBP-exposed testes including denudation of germ cells, vacuolization of Sertoli cell cytoplasm, thickening of lamina propria of seminiferous tubules, and focal lymphocytic infiltration. Entire sections of testes containing almost exclusively mature spermatozoa were found in 1.0, 5.0, and 10.0 ppm DBP-exposed testes, indicating impairment of spermiation. Testicular hypoplasia and seminiferous tubular dysgenesis were also evident in DBP-treated frogs. | Lee and Veeramachaneni, 2005 |
| **Type/Objective:** Recent in vitro studies have shown that DBP and mono-n-butyl phthalate (MBP), the major metabolite of DBP, possessed thyroid hormone receptor (TR) antagonist activity. It is therefore important to consider DBP and MBP that may interfere with thyroid hormone system.<br>**Conclusion:** The current findings highlight potential disruption of thyroid signalling by DBP and MBP and provide data for human risk assessment.<br>**Method:** Nieuwkoop and Faber stage 51 Xenopus laevis were exposed to DBP and MBP (2, 10 or 15 mg/L) separately for 21 days. | Shen et al, 2011 |

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| **Results:** The two test chemicals decelerated spontaneous metamorphosis in X. laevis at concentrations of 10 and 15 mg/L. Moreover, MBP seemed to possess stronger activity. The effects of DBP and MBP on inducing changes of expression of selected thyroid hormone response genes: thyroid hormone receptor-beta (TRβ), retinoid X receptor gamma (RXRγ), alpha and beta subunits of thyroid-stimulating hormone (TSHα and TSHβ) were detected by qPCR at all concentrations of the compounds. Using mammalian two-hybrid assay in vitro, we found that DBP and MBP enhanced the interactions between co-repressor SMRT (silencing mediator for retinoid and thyroid hormone receptors) and TR in a dose-dependent manner, and MBP displayed more markedly. In addition, MBP at low concentrations (2 and 10 mg/L) caused aberrant methylation of TRβ in head tissue. | |
| **Type/Objective:** Phthalate ester plasticizers are antiandrogenic in mammals. Given the similarity between mammalian and teleost endocrine systems, phthalate esters may be able to cause antiandrogenic endocrine disruption in fish in the wild.<br>**Conclusion:** These results suggest that DBP has antiandrogenic effects in fish.<br>**Method:** In the present study, adult male three-spined sticklebacks (Gasterosteus aculetaus; n = 8) were exposed to DBP at 0, 15, and 35 µg DBP/L for 22 d and analyzed for changes in nesting behavior, plasma androgen concentrations, spiggin concentrations, and steroidogenic gene expression.<br>**Results:** Plasma testosterone concentrations were significantly higher in males from the 35 µg DBP/L group compared with the solvent control, whereas plasma 11-ketotestosterone concentrations were not significantly affected. Expression of steroid acute regulatory protein and 3β-hydroxysteroid dehydrogenase remained unchanged. Spiggin concentrations were significantly lower in the males exposed to 35 µg DBP/L. Nest building appeared to be slower in some males exposed to DBP, but this was not statistically significant. | Aoki et al, 2011 |
| **Type/Objective:** This study investigated cytotoxicity, endocrine disruption, effects mediated via AhR, lipid peroxidation and effects on expression of enzymes of xenobiotic metabolism caused by DEHP, DEP, DBP and BBP in developing fish embryos.<br>**Conclusion:** The study highlights the need for simultaneous assessment of: (1) multiple cellular targets affected by phthalates and (2) phthalate mixtures to account for additive effects when multiple phthalates modulate the same pathway.<br>**Method and Results:** Oxidative stress was identified as the critical mechanism of toxicity (CMTA) in the case of DEHP and DEP, while the efficient removal of DBP and BBP by phase 1 enzymes resulted in lesser toxicity. DEHP and DEP did not mimic estradiol (E(2)) in transactivation studies, but at concentrations of 10mg/L synthesis of sex steroid hormones was affected. Exposure to 10mg BBP/L resulted in weak transactivation of the estrogen receptor (ER). All phthalates exhibited weak potency as agonists of the aryl hydrocarbon receptor (AhR). The order of potency of the 4 phthalates studied was; DEHP>DEP>BBP>>DBP. | Mankidy et al, 2013 |
| **Type/Objective:** Investigation of endocrine effects of DnBP in female fish.<br>**Conclusion:** These data show that a continuous exposure to subacute concentrations of DnBP for 7 d can cause antiestrogenicity in female adult Murray rainbowfish.<br>**Method:** The present study investigated the changes in ovarian histology and serum vitellogenin concentrations in adult Murray rainbowfish after exposure to 125 µg/L, 250 µg/L, 500 µg/L, and 1000 µg/L DnBP for 7 days.<br>**Results:** Treatment at 125 µg/L to 1000 µg/L DnBP for 7 d had no significant effect on the survival, condition factor, gonadosomatic index, hepatosomatic index, and developmental stage of the fish. Based on the histological investigation, the sizes of the previtellogenic oocytes in the fish treated at 250 µg/L to 1000 µg/L were found to | Bhatia et al, 2013 |

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| be significantly higher than in the corresponding control fish ($p \leq 0.05$). The early vitellogenic oocytes in the fish treated at 1000 µg/L were significantly smaller relative to those in the unexposed fish ($p \leq 0.05$). Histological changes like chorion folding, shrunken ooplasm, impaired yolk production, granulomatous inflammation, and interstitial fibrosis were observed in the ovaries of the fish treated with DnBP. The circulating levels of plasma vitellogenin were significantly lower in the fish exposed to 500 µg/L and 1000 µg/L DnBP ($p \leq 0.05$). | |
| **Type/Objective:** Evaluation of endocrine disrupting effects of DBP in fish.<br>**Conclusion:** Collectively, an increase in the proportion of spermatogonia in the testes, the upregulation of the genes for the oestrogen receptors and choriogenin in the liver, an induction in the brain aromatase activity and the increase in the circulating levels of plasma vitellogenin suggest that continuous exposures for 7 days to sub-acute concentrations of DnBP can adversely affect the reproductive health of the male Murray rainbowfish by an estrogenic mode of action.<br>**Method:** This study investigated the effects of 7-day exposures to nominal (measured) concentrations of 125 (62), 250 (140), 500 (230) and 1,000 (383) µg/L of DnBP on the biomarkers of reproduction in adult male Murray River rainbowfish (Melanotaenia fluviatilis) using molecular, biochemical and histological endpoints.<br>**Results:** None of the tested concentrations of DnBP had any effect on survival or the vital body indices of the fish. The sizes of spermatogonia, Type A and B spermatocytes and spermatids were significantly smaller relative to the controls after treatment with DnBP. This was accompanied by a significant increase in the proportion of spermatogonia in fish treated with 250-1,000 µg/L of DnBP in comparison to the unexposed fish. At the end of the exposure period, the expressions of the transcripts for the androgen receptors α and β were significantly elevated in the livers of the fish treated with 500 and 1,000 µg/L of DnBP. In addition, there was also an increase in the circulating concentrations of vitellogenin in the plasma in the higher treatment groups. An induction in the activity of aromatase was noted in the brains of 1,000 µg/L DnBP-treated fish. This was accompanied by an increase in the hepatic expression of the genes (here and later, whenever the phrase gene expression is used as a synonym for gene transcription although it is acknowledged that it is also regulated, e.g., by translation, mRNA stability and protein stability) encoding for the oestrogen receptors α and β and choriogenin L. | Bhatia et al, 2014 |
| **Type/Objective:** To evaluate the combined effects of 17α-ethinyl estradiol (EE2) and DBP on zebrafish (Danio rerio) from the juvenile state to the adult stage.<br>**Conclusion:** However, the influence on morphology of gonad, liver, and gill induced by exposure to the mixture of EE2 and DBP was generally more potent than that by single exposure to EE2 or DBP, indicating the combined long-term harmful effects of EE2 and DBP on the development of zebrafish.<br>**Method:** The authors spiked EE2 (5 ng/L and 20 ng/L) and DBP (0.1 mg/L and 0.5 mg/L) either individually or in mixture.<br>**Results:** At 45 d postfertilization (dpf), the survival rate of zebrafish was comparable in all treatments. DBP did not induce vitellogenin (VTG) synthesis, and no interaction was found between EE2 and DBP on VTG induction. At 90 dpf, both liver and gill were subject to more severe damage (lipid vacuoles of hepatocytes, amalgamation of gill lamellae, and clubbing at the tips of the secondary lamellae) when coexposed to these 2 chemicals, compared with single exposure. At 115 dpf, generally none of the binary mixture groups showed significantly different growth and sex ratios compared with the corresponding EE2 alone groups. In conclusion, no obvious interactions were detected between EE2 and DBP on the growth, VTG induction, or sex ratio of zebrafish, and they may act independently. | Chen et al, 2015 |

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| **Type/Objective:** To evaluate the effects of DBP on abalone<br>**Conclusion:** The results revealed that DBP may lead to abalone oxidative stress, lipid metabolism dysfunction, energy metabolism disturbance, and osmoregulation imbalance.<br>**Method:** Marine gastropods (abalone) were exposed to DBP at environmentally relevant concentrations (2, 10, and 50 μg/L) for 30 days. The plasma metabolite profiles were determined at the 5th, 15th, and 30th.<br>**Results:** The major metabolite changes corresponding to DBP exposure were related to osmotic regulation, energy metabolism, and environmental stress, and the effects displayed a dose-dependent pattern. The most obvious change was the increase in the levels of intracellular metabolites (betaine, dimethylglycine, homarine, glutamine, and lactate) and tricarboxylic acid cycle intermediates. | Zhou et al, 2015 |
| **Type/Objective:** To evaluate the combined effects of 17α-ethynylestradiol (EE2) and dibutyl phthalate (DBP) on the growth and reproduction of male zebrafish<br>**Conclusion:** Our findings indicate that the effects of mixed EE2 and DBP at environmentally relevant levels can be either antagonistic or additive relying on the specific toxicological endpoints and the respective doses of each chemical.<br>**Method:** Three-month-old fish were exposed to 0.005 or 0.020μg/L EE2, 100 or 500μg/L DBP or their binary mixtures under semi-static conditions. Investigated parameters include the length, weight, condition factor, vitellogenin (VTG) induction, acyl-CoA oxidase (AOX) protein level, histopathological alteration of testis, liver and gill, and reproductive capacity.<br>**Results:** After 21d exposure, no statistical difference was found among the weights, lengths and condition factors of different treatment groups. In all binary mixture groups, decreased VTG levels were detected compared to EE2-only groups; and the AOX levels were significantly lower than DBP-only treatments while both chemicals can individually induce AOX synthesis. Therefore, EE2 and DBP may act additively on VTG and antagonistically on AOX induction in males. After 45d exposure, delayed gametogenesis was observed for the DBP-only groups, indicated by fewer spermatozoa and more spermatocytes, which was further aggravated with the addition of EE2. The developmental delay of testis partially recovered after a 30d depuration in clean water. Combined exposure also caused liver and gill lesions, which were not alleviated during the 30d depuration, suggesting a nonreversible harmful effect the same as single exposure. Mixed EE2 and DBP were observed to impair the reproductive capability (the fecundity and fertilization rate) of males, while single exposure did not. Co-exposed to 0.020μg/L EE2 and 100μg/L DBP promoted the early hatching of offspring (F1 generation) at 48h post-fertilization (hpf), but the survival rates of the F1 generation were similar in all treatments. | Xu N et al, 2014 |

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