

Organotins and Phthalates
(version updated January 5, 2011)

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1.1 Epidemiology

1.1.1 Organotins

There are no epidemiology studies available that evaluate the association of organotin exposure with diabetes, adiposity, or health effects related to metabolic syndrome. There are poisoning incident reports, mostly in workers involved in applying the compounds for pesticide use, that note observations of hyperglycemia and/or [glycosuria](#) [(Colosio *et al.* 1991; Manzo *et al.* 1981), reviewed in NIOSH Criteria Document for Organotin Compounds (1976)]. One of the more detailed accounts is a poisoning case report by Colosio *et al.* (1991) describing slight glucose intolerance in a 36-year old man who spilled a package of a pesticide powder containing 18.95% triphenyltin (TPT) acetate on his arms. His blood glucose was normal upon admission to the hospital (113 mg/dL) but he developed impaired glucose tolerance and slight glycosuria by the 5th day after the incident. Tin concentrations in the blood on the 5th day were ~50 $\mu\text{g/l}$, down from the initial concentration of 80 $\mu\text{g/l}$ measured on the second day.

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1.1.2 Phthalates

Two cross-sectional studies published in the peer-reviewed literature have used data from NHANES 1999-2002 to assess associations between urinary levels of phthalate metabolites and indicators of obesity or insulin resistance (Hatch *et al.* 2008; Stahlhut *et al.* 2007) (Appendix Table A). The study by Stahlhut *et al.* (2007) focused on phthalate metabolites in men aged >20 years and waist circumference and **HOMA** as an indicator of insulin resistance. Four phthalate metabolites were associated with increased waist circumference (MEP, MBzP, MEHHP, and MEHOP) and two with insulin resistance (MEP, MBzP) in the fully adjusted model.¹ Inclusion of an adjustment variable for renal function (glomerular filtration rate, or GFR) was described as having minimal impact on the reported associations (data not shown). Inclusion of the liver enzymes alanine aminotransferase (ALT) and γ glutamyl transpeptidase (GGT) in the regression model had more of an impact. For example, MBP was associated with insulin resistance [β (SE) = 0.064(0.024); p=0.011] in a reduced model without these variables.² The association was attenuated when measures of liver enzyme activity were added to the model, [β (SE) = 0.043(0.023); p=0.081]. It is unclear whether statistical adjustment for liver enzymes is appropriate. It would be most appropriate to adjust for liver enzymes if they are altered by the health condition of obesity in a manner that results in altered phthalate metabolism. Conversely, it would be less appropriate to adjust for liver enzyme levels if they are directly affected by phthalate exposure.

Phthalate acronyms	
phthalate	urinary metabolite
BBzP : benzylbutyl phthalate	MBzP : monobenzyl phthalate
DBP: dibutyl phthalates	MButP or MBP : Mono-n-butyl phthalate MiBP : mono-isobutyl phthalate
DCHP : dicyclohexyl phthalate	MCHP : mono-cyclohexyl phthalate
DEHP : di-2-ethylhexyl phthalate	MEHP : mono-2-ethylhexyl phthalate MEHHP : mono(2-ethyl-5-hydroxyhexyl) phthalate MEOHP : mono(2-ethyl-5-oxohexyl) phthalate MECPP : mono(2-ethyl-5-carboxypentyl) phthalate
DEP : diethyl phthalate	MEP : monoethyl phthalate
DiNP : di-isononyl phthalate	MiNP : mono-isononyl phthalate
DMP : dimethyl phthalate	MMP : monomethyl phthalate
DOP : di-n-octyl phthalate	M CPP : mono (3-carboxypropyl) phthalate MOP : Mono-n-octyl phthalate

(CDC 2009)

Hatch *et al.* (2008) also used data from NHANES 1999-2002 to look for associations between urinary levels of phthalate metabolites and waist circumference and BMI in 4,369 subjects. This study differed from the study by Stahlhut *et al.* (2007) because it included women in the analysis, conducted separate analyses for children, adolescents, and adults (6-11 years, 12-19

¹ Adjusted for age, race/ethnicity, total fat and calorie intake, physical activity level, smoking exposure, urine creatinine, glomerular filtration rate, ALT, and GGT.

² Adjusted for age, race/ethnicity, total fat and calorie intake, physical activity level, smoking exposure, and urine creatinine.

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years, 20-59 years, and 60-80 years), and adjusted for different variables³. The linear regression models examined associations across age/gender specific exposure quartiles. Different patterns of associations were seen among men and women with the strongest positive associations with waist circumference or BMI seen in men aged 20-59 for MBzP, MEHHP, and MEOHP. The findings for females varied by metabolite, with some positive associations for MEP, especially among adolescents for both waist circumference and BMI; however, inverse associations were seen with MBP in 60-80 year olds (waist circumference and BMI) and MEHP and BMI in females 12 years of age and above. Patterns of association for MBzP, MEP and MEHHP and indicators of obesity from these the NHANES studies are presented in [Figure 2](#).

A third epidemiology report was presented in a poster session at the 2010 International Union of Toxicology (IUTOX) meeting (Svensson *et al.* 2010). This report described associations between urinary levels of phthalate metabolites and BMI, waist circumference, waist height ratio, and diabetes in 221 Mexican women participating in an ongoing case-control study for breast cancer. The adjusted odds ratios for self-reported diabetes were significantly reduced for MBzP and borderline elevated for Σ DEHP and MECPP ([Figure 1](#)). The only phthalate metabolite that was associated with the obesity indicators was MEHHP ($r_{\text{waist circumference}} = 0.15$; $r_{\text{waist height ratio}} = 0.20$).

Figure 1. Associations between urinary levels of phthalate metabolites and diabetes prevalence in 221 Mexican women

phthalate metabolite (parent phthalate)	Adjusted* OR (95% CI) for log transformed urinary phthalates and self-reported diabetes (n=221, 39 cases/182 non-cases)
MEP (DEP)	1.02 (0.74-1.39)
MBP (DBP)	1.10 (0.75-1.61)
MiBP (DiBP)	1.01 (0.65-1.55)
MBzP (BBzP)	0.74 (0.55-1.00)*
MCPP (DOP)	—
DEHP metabolites	
MEHP	1.01 (0.68-1.49)
MEHHP	1.40 (0.84-2.33)
MEOHP	—
MECPP	1.54 (0.92-2.55)
Σ DEHP	1.64 (0.98-2.73)

* Adjusted by: creatinine and education (MEP, MBP, MiBP, MBzP, and MEHP); creatinine and age (MEHHP and MECPP); creatinine and waist-height ratio (MEHHP and Σ DEHP)

** $p < 0.05$

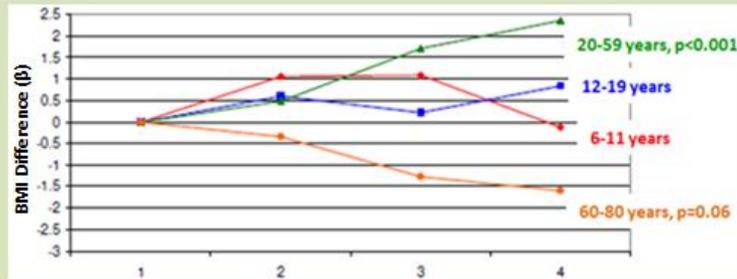
From Svensson *et al.* (2010)

³ Hatch *et al.* (2008) adjusted for age, race/ethnicity, creatinine, height, SES, dietary factors (e.g., energy intake, fiber intake, macronutrient intake), TV/video/computer use, physical activity (age 12+), smoking (age 20+), and reproductive factors (menopause status and parity in females age 20+).

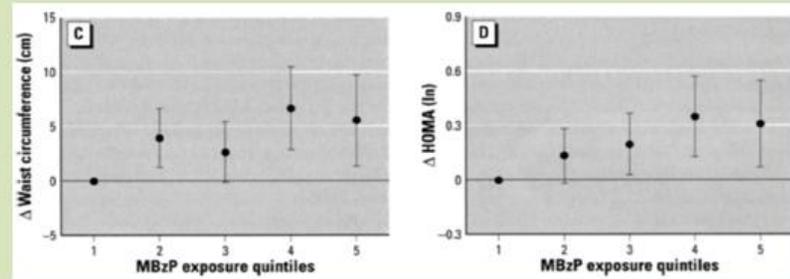
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Figure 2. Patterns of association between urinary levels of phthalate metabolites and indicators of obesity or insulin resistance in NHANES 1999-2002.

Strongest association with MBzP is in adult males, aged 20-59

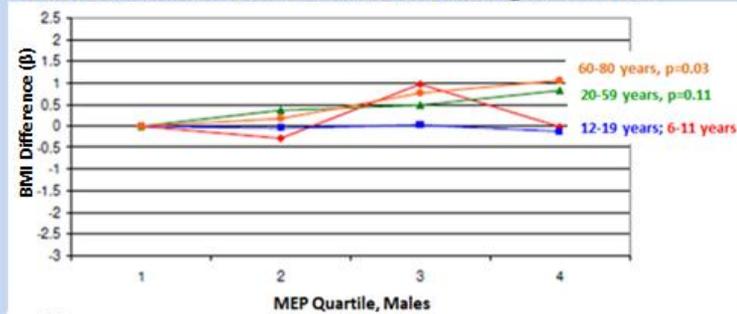


MBzP exposure quartiles, males (Hatch et al. 2008)

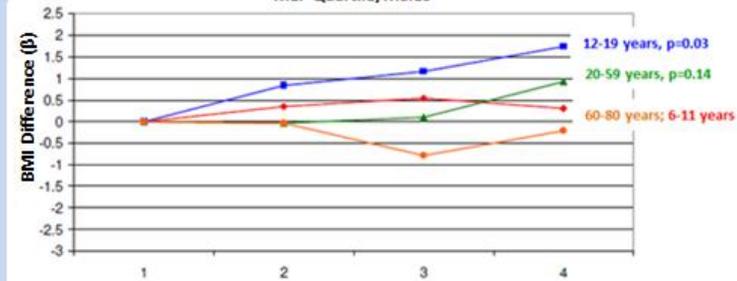


MBzP, males aged >20 years (Stahlhut et al. 2007)

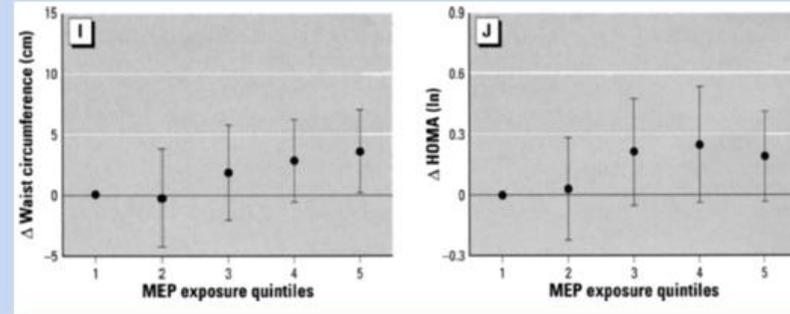
Positive associations in men and adolescent girls with MEP



MEP Quartile, Males

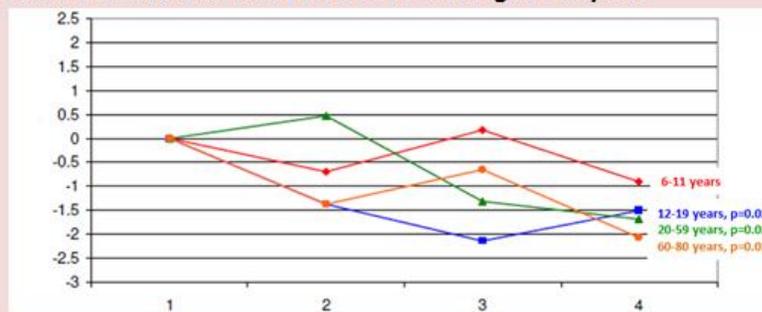


MEP Quartile, Females
MEP (Hatch et al. 2008)



MEP, males aged >20 years (Stahlhut et al. 2007)

Inverse associations with MEHP in females aged >12 years



MEHP exposure quartiles, females (Hatch et al. 2008)

1.2 Animal and Mechanistic Studies

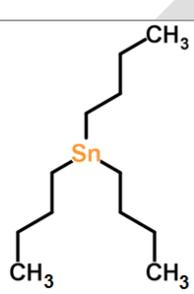
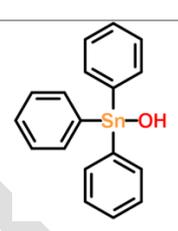
1.2.1 Organotins

Organotins are now primarily used as stabilizers in plastics manufacturing. These compounds are well-established immunotoxicants as evaluated in laboratory animal studies (De Waal *et al.* 1993; Luebke *et al.* 2006; Vos *et al.* 1990; Vos *et al.* 1984) and are now also being referred to as developmental “obesogens” (Chen *et al.* 2009; Grun and Blumberg 2009). This is based on studies conducted in the past decade that report stimulatory effects of tributyltin (TBT) on adipocyte differentiation (*in vitro* and *in vivo*) and increased amount of fat tissue in adult animals exposed to TBT during fetal life (Grun and Blumberg 2006; Hiromori *et al.* 2009; Inadera and Shimomura 2005; Kanayama *et al.* 2005; Kirchner *et al.* 2010; Nakanishi *et al.* 2005). *In vitro* effects of TBT include increased lipid accumulation in adipocytes and the promotion of

multipotent stromal stem cells to differentiate into adipocytes. Mice exposed to TBT *in utero* also show more lipid accumulation in adipocytes as neonates and have more fat mass in adulthood compared to control animals (i.e., larger epididymal fat pads). The biological plausibility of the findings is supported by TBT’s potent PPAR γ and RXR agonistic activities [Table 1; (le Maire *et al.* 2009; Nakanishi 2008; Nishikawa *et al.* 2004)]. In addition to the studies of TBT, an older literature describes hyperglycemic effects of another organotin, triphenyltin (TPT), in adult animals (Manabe and Wada 1981; Matsui *et al.* 1984; Ogino *et al.* 1996; Ohhira *et al.* 1999, 2000; Watanabe *et al.* 2002). These studies are discussed in more detail below and summarized in Appendix Table B.

Adipocyte differentiation and adiposity

Figure 3. Examples of organotin compounds

Tributyltin (TBT)	Triphenyltin (TPT) hydroxide
	(Fentin)
1461-22-9 (TBT chloride)	76-87-9
$C_{12}H_{28}Sn$ (MW 291.1)	$C_{18}H_{16}OSn$ (MW 367.0)
	
TBT uses include wood preservation, antifouling pesticide in marine paints, antifungal action in textiles and industrial water systems, such as cooling tower and refrigeration water systems, wood pulp and paper mill systems, and breweries. TBT oxide is the most widely used compound in TBT-containing commercial products. TBT chloride is a common form used in toxicology studies. There primary use has been as stabilizers in plastics in manufacturing.	TPT hydroxide (Fentin) is used as a fungicide for potatoes, sugar beets, and pecans. It was first registered for use as a pesticide in the United States in 1971. *High throughput screening results from ToxCast™ in Appendix Table G

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A number of *in vitro* studies demonstrate that organotins activate PPAR γ and RXR and stimulate adipocyte differentiation *in vitro* (Hiromori *et al.* 2009; Inadera and Shimomura 2005; Kanayama *et al.* 2005; le Maire *et al.* 2009; Nakanishi *et al.* 2005; Nishikawa *et al.* 2004). These findings were extended by Grun *et al.* (2006) to include *in vivo* assessment. Like others, Grun *et al.* found TBT to be a potent agonist for PPAR γ and RXR (Table 1). *In vitro*, TBT induced adipogenesis in 3T3-L1 cells treated with an adipogenic sensitizing cocktail⁴ at concentrations of 10 and 100 nM (Figure 4A). Similar effects were seen with 10 and 100 nM AGN195203 (a synthetic RXR-specific ligand) and 1 and 10 μ M troglitazone (a PPAR γ -agonist), but not the RAR agonist TTNPB [(E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthylenyl)-1-propenyl]].

Grun *et al.* (2006) also reported *in vivo* effects on adipocyte differentiation in neonatal C57BL/6 mice that were treated *in utero* by ip injection to the dam with 0, 0.05 or 0.5 mg/kg bw/day TBT from gestational days 12-18. Histological evaluation indicated signs of **steatosis** in the liver consistent with dysregulation of fatty acid uptake. Oil Red O positive staining in mammary and inguinal adipose tissues was elevated, reflecting either an increase in lipid accumulation or an increase in mature adipocytes (Figure 4B). A separate group of mice were similarly treated *in utero* and assessed at 6 weeks of age. In males, the weight of the epididymal fat pad was 1.20-fold larger compared to control animals but the animals weighed significantly less. As the animals aged, the degree of adiposity increased and they became heavier than control animals (personal communication with Bruce Blumberg, October 3, 2010). TBT was also reported in Grun *et al.* (2006) to affect adipocyte development in *Xenopus laevis* larva treated with 1-10 nM TBT from stage 48 to metamorphosis. A dose dependent increase in ectopic adipocyte formation posterior to the fat bodies in and around the gonads was seen in both sexes. Concentrations of 10 nM TBT, 10 nM AGN195203 (RXR-specific ligand), and 1 μ M troglitazone (PPAR α agonist) produced ectopic adipocytes in ~45-60% animals (body weight not reported).

Table 1. Organotin EC₅₀ values for nuclear receptor ligand binding domains (in μ M)

	hRXR α	hRAR α	hPPAR γ *
9-cis retinoic acid	15	not active	not active
all trans retinoic acid	not active	8	not active
AGN195203	0.5-2	not active	not active
troglitazone	not active	not active	1000
butyltin chloride	not active	not active	not active
dibutyltin chloride	3000	not active	not active
tributyltin (TBT)	3-8	not active	20
tetrabutyltin	150	ND	ND
di(triphenyltin) oxide	2-10	not active	20
butyl tris(2-ethylhexanoate)	not active	ND	ND
TBT fluoride	3	ND	ND
TBT bromide	4	ND	ND
TBT iodide	4	ND	ND
triethyltin bromide	2800	ND	ND
trimethyltin chloride	>1000	ND	ND

ND, not determined. EC₅₀ values were determined from dose-response curves of GAL4-NR LBD construct activation in transiently transfected Cos7 cells after 24-hour ligand exposure.

*Many of these compounds were also assessed for PPAR α and were general not active (personal communication with Bruce Blumberg, November 28, 2010)

AGN195203 = a synthetic RXR-specific ligand

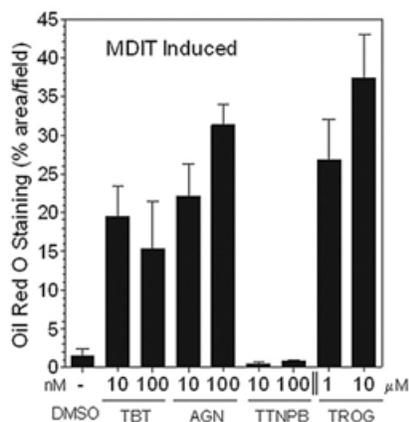
Reprinted from Grun *et al.* (2006) with permission from publisher.

⁴ MDIT, an adipogenic sensitizing cocktail containing IBMX (3-isobutyl-1-methylxanthine), dexamethasone, insulin and triiodothyronine (T3)

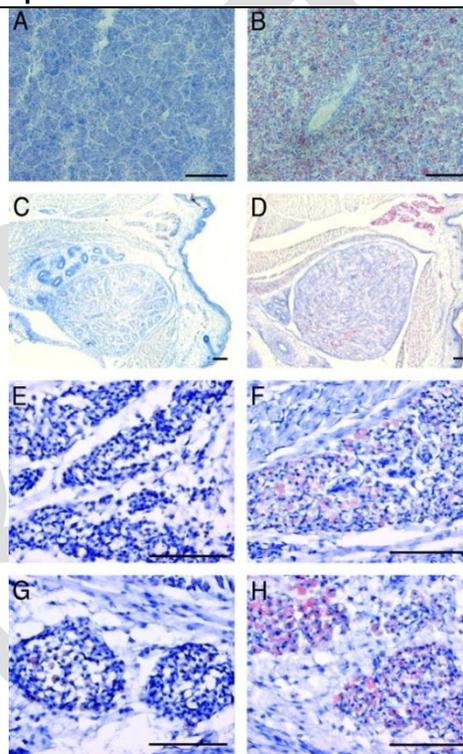
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Further studies from this research group showed that TBT exposure could stimulate human and mouse multipotent stromal stem cells derived from white adipose tissue (adipose-derived stromal stem cells, ADSCs) to favor the production of adipocytes (Kirchner *et al.* 2010). Adipogenesis was increased in mouse and human ADSCs treated with 50 nM TBT or 500 nM rosiglitazone (ROSI), a highly specific PPAR γ 2 agonist known to induce adipogenesis in multipotent stromal cell/mesenchymal stem cells and other cell culture models. Specific findings in Kirchner *et al.* (2010) included increased lipid accumulation and an increase in the percent of cells with lipid that were stained with Oil Red O (Figure 5).

Figure 4. TBT induces (A) adipogenesis in 3T3-L1 cells *in vitro* and (B) *in utero* exposure to tributyltin (TBT) increases adiposity in mouse liver, testis, and adipose depots *in vivo*



2A. MDIT-induced 3T3-L1 cultures were grown for 1 week in the presence of vehicle (DMSO) or ligands and analyzed for mature adipocyte differentiation by Oil Red O staining. The percentage area stained was determined by automated analysis of random fields (n = 9) from high-contrast dissecting scope photographs of monolayers analyzed in Image J. * Reprinted from Grun *et al.* (2006) with permission from publisher.



2B. Histological sections (12 μ m) of newborn mouse liver (A and B), testis (C and D), inguinal adipose (E and F) and mammary adipose (G and H) stained with Oil Red O and counterstained with hematoxylin following *in utero* exposure to vehicle only (sesame oil) (A, C, E, and G) or 0.5 mg/kg b.w. TBT (B, D, F, and H) given s.c. daily from E12–18. Scale bar, 100 μ m.
* Reprinted from Grun *et al.* (2006) with permission from publisher.

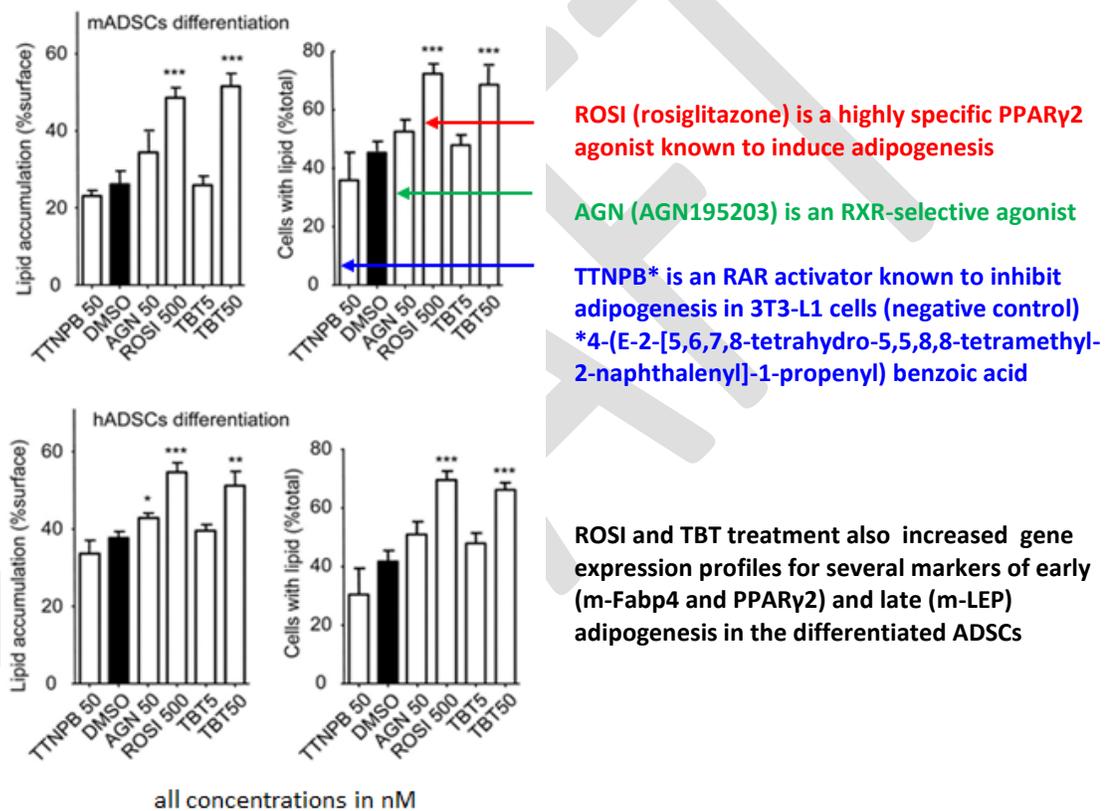
The RXR-selective agonist AGN195203 exhibited weaker effects on adipocyte differentiation at 50 nM, i.e., increases in lipid measurements were observed but they were generally not statistically significant. Gene expression profiles of the adipogenesis induced mouse ADSCs and human ADSCs indicated increased expression in TBT and ROSI-treated cells for several markers of early (m-Fabp4 and PPAR γ 2) and late (m-LEP) adipogenesis.⁵ Co-treatment of mADSCs with concentrations of the PPAR γ 2 antagonist T007097 as low as 100 nM blocked the adipogenic

⁵ Gene expression of the early marker Pref-1 was decreased in both mADSCs and hADSCs by treatment with 500 nM ROSI or 50 nM TBT. The late marker ADIPOQ was not affected except in the 500 nM ROSI treatment in hADSCs.

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effects of ROSI and TBT, indicating the importance of PPAR γ activation in mediating the adipogenic effects. TBT was more potent than ROSI in enhancing adipogenesis (50 nM versus 500 nM). The authors suggested this could be because TBT is a ligand for both RXR and PPAR γ . Proliferation assays showed dose-dependent reductions in proliferation for ROSI (tested at 10, 100, 500 nM) and TBT (tested at 5, 10, 100 nM), including significantly less proliferation at the same concentrations of ROSI and TBT that stimulated adipocyte differentiation based on lipid analysis of the cells, 500 nM ROSI and 50 nM TBT. The results for both lipid-related measures and proliferation assays were very similar in mADSCs and hADSCs.

Figure 5. *In vitro* effect of tributyltin (TBT) exposure on adipogenic capacities of adipose-derived stromal stem cells (ADSCs)



Adipogenesis was induced in mouse and human ADSCs (mADSCs, hADSCs) by the addition of an adipogenic cocktail for 14 d in the absence (DMSO) or presence of nuclear receptor agonists 50 nM TTNPB (negative control RAR activator), 50 nM AGN (RXR-selective agonist), 500 nM ROSI (PPAR γ -specific agonist), or two doses of TBT (5 and 50 nM) (n=3 wells per treatment). Undifferentiated cells were kept in basic MSCs expansion media, able to prevent differentiation, as a negative control (untreated cells). Lipid accumulation was stained by Oil Red O and quantified with Image J software. The number of cells with lipid droplets was also visually counted. Reprinted from figures 2A and 2C of Kirchner et al. (2010) with permission from publisher.

Kirchner et al. (2010) conducted parallel studies to see if TBT and ROSI caused similar adipogenic effects in ADSCs isolated from mice that were prenatally exposed on GD 16.5 to 0.1 mg/kg bw TBT or 1 mg/kg bw ROSI via gavage feeding to the dam. ADSCs were isolated from

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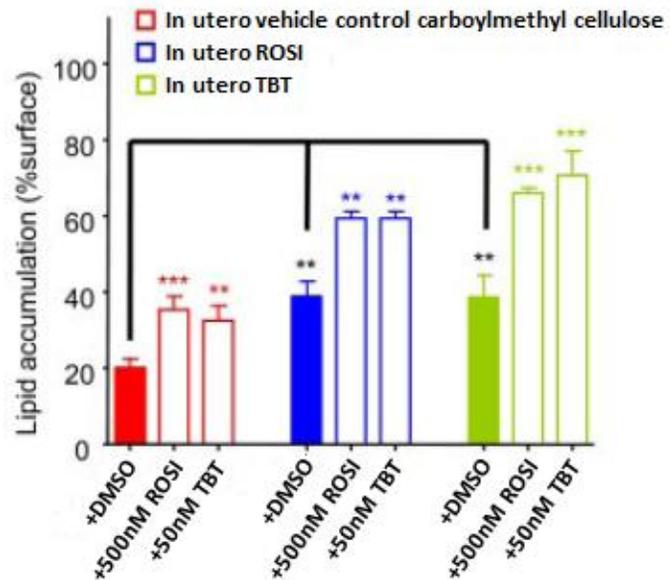
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the mice at 8 weeks of age and treated *in vitro* with DMSO, 50 nM TBT, or 500 nM ROSI. Lipid accumulation was significantly increased in mADSCs isolated from TBT- or ROSI-treated animals, even when the *in vitro* treatment was DMSO (Figure 6). Kirchner et al. (2010) also showed an increase in the number of preadipocytes in the prenatally-treated TBT animals. The magnitude of the increases was largest when the *in vitro* treatment was ROSI or TBT. The bone differentiation capacity of ADSCs treated with an osteogenic cocktail was reduced by 2- and 2.8-fold following prenatal ROSI or TBT treatment. The mRNA levels of fatty acid-binding protein 4 (Fabp4) mRNA, an early marker of adipogenesis, was increased about 2-fold in the TBT-treated animals and the promoter/enhancer regions of this gene were hypomethylated in the isolated ADSCs from these animals, suggesting epigenetic modifications. Carfi et al. (2008) also reported that 0.001 or 0.01 μ M TBT treatment to human bone

marrow cultures increased the expression of the adipocyte differentiation marker, aP2, or adipocytes-specific fatty acid binding protein-2, and PPAR γ (but not RXR). At the same time, TBT inhibited several haematopoietic factors involved in leukocyte differentiation, including leptin.

Only one study has assessed the impacts of TBT on leptin or the adipose-secreted proteins [adiponectin](#) and [resistin](#). Zuo et al. (2009) treated postnatal day (PND) 21 male KM mice with TBT chloride by oral gavage once every three days at 0.0005, 0.005, or 0.05 mg/kg bw over a 45-day period. The most consistent findings from this study were increases in plasma leptin levels of ~1.3 to 1.6-fold and decreases in hepatic adiponectin to ~72 to 87% of control levels in all TBT-treated groups. The increases in plasma leptin did not necessarily correspond to significant differences in the peripheral fat mass (renal+testicular)/body weight ratio or body weight gain, which were only increased in animals treated with 0.005 mg/kg bw. Effects of TBT on the plasma levels of other adipose-secreted proteins, adiponectin and resistin, were less

Figure 6. Effect of prenatal TBT exposure on adipogenic capacities of mADSCs



Lipid accumulation in ADSCs isolated from 8-week old C57BL/6J mice that were prenatally treated on GD 16.5 with vehicle control carboxymethyl cellulose (CMC), 0.1 mg/kg TBT, or 1 mg/kg ROSI via gavage feeding to the dam. The mADSCs harvested from exposed animals were additionally treated *in vitro* with 50 nM TBT, 500 nM ROSI, or a DMSO vehicle control, in the presence of an adipogenic induction cocktail (n = 3 wells per treatment in triplicates) for 14 d. Undifferentiated cells were kept in basic MSCs expansion media, able to prevent differentiation, as a negative control (untreated cells). A, Lipid accumulation was stained by Oil Red O and quantified by Image J software. Reprinted from Figure 5A of Kirchner et al. (2010) with permission from publisher.

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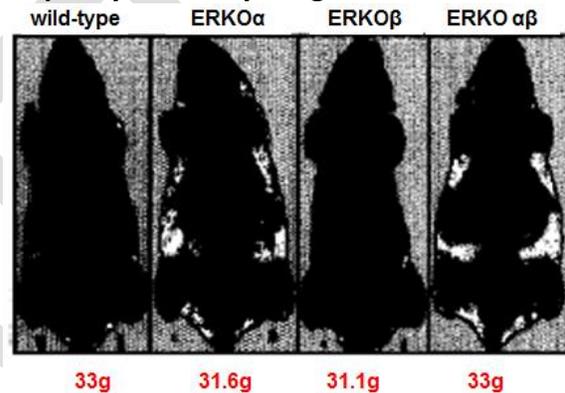
consistent across the dose levels tested and there was a general trend of increased serum insulin that was only statistically significant in the 0.005 mg/kg/bw group. The TBT treatment also caused severe hepatocyte cytoplasmic degeneration and hepatic steatosis, “fatty liver,” where the bulk of lipid droplets in the liver tissue increased as the TBT doses increased.

Higher doses of fentin of ~1 to 7 mg/kg bw/d typically cause decreased body weight in laboratory rodents,⁶ although a recent study by Si et al. (2010) reported increased body weight in male KM mice treated with TBT chloride by ip injection at 0.05 mg/kg bw but not 0.5 mg/kg body weight. In general, body weight findings may have limited utility for addressing whether organotins or other environmental chemicals alter adiposity. It is general accepted that body weight is a relatively crude indicator of internal body fat in rodent models. For example, no changes in body weight were observed by Ohlsson et al.(2000) in estrogen receptor α knockout mice (ERKO α) mice compared to wild-type controls despite having visibly greater amounts of adipose tissue (Figure 7). Adiposity was imaged using dual X-ray absorptiometry.

Glucose homeostasis and pancreatic effects

Triphenyltin (TPT) was suggested for use as a diabetogenic agent in animal models by Manabe and Wada in 1981 (Manabe and Wada 1981). The initial observations were decreased fasting insulin and increased fasting glucose, triglycerides, total cholesterol and phospholipid in rabbits treated with single high dose of TPT fluoride (100 mg/kg bw by oral gavage; 25% of LD₅₀) and followed for a period of 21 days. Levels of insulin, glucose, and lipids were no different from control values by 10 days post-dosing. Insulin release was blunted in TPT-treated animals that underwent IV glucose, glucagon, and arginine challenge studies. Glucagon release was also reduced in animals during the arginine challenge. Plasma glucose was elevated in the TPT- fluoride treated animals in all the IV challenge studies. The authors also noted very large reductions in plasma and adipose LPL activity to ~16% and ~10% of control levels, respectively. This effect was most apparent 3-7 days after the last dose. No histological abnormalities were noted in the islet cells. Based on these findings, the authors concluded that TPT-fluoride induces insulin-deficient state and diabetic hyperlipidemia in rabbits. The diabetogenic profile caused by TPT-fluoride differed from that caused by streptozotocin or alloxan because it was transient and occurred in the

Figure 7. Lack of relationship between adiposity and body weight in ERKO α mice.



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⁶ Based on ToxRef findings for subchronic and chronic studies in rats and/or mice (Suter 1986), and chronic (Tennekes *et al.* 1989) rat and mice (Tennekes 1989). Effects on reduced weight of litter, or pup growth were reported in a multigeneration study at the highest dose tested, 3.9 mg/kg/day in the diet (American Hoechst Corporation 1986),

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absence of islet cell damage. Note that the interpretation of this study is complicated by the use of the fluoride salt of TPT. Similar inhibitory effects of acute oral administration of 40 $\mu\text{mol}/100\text{ g NaF}$ on plasma insulin levels and resultant glycemia in rats were reported by Rigalli et al. (1990). Values returned to normal 4-5 hours following dose administration (7.6 mg/kg compared to 5.4 mg/kg F delivered as TPT-fluoride to rabbits by Manabe et al).

No additional studies appeared in the peer-reviewed literature until later when a collection of reports by a Japanese research group were published between 1984 and 2002 reporting hyperglycemia in animals treated with high doses of TPT chloride (60-200 mg/kg) (Matsui et al. 1984; Ogino et al. 1996; Ohhira et al. 1996; Ohhira et al. 1999, 2000; Watanabe et al. 2002). More recent publications have focused on the adipogenic effects of TBT at relatively low doses (discussed above) and additional findings related to TPT-induced hyperglycemia have not been reported. A 2009 study by Zuo et al. (2009) reported no effect on fasting plasma glucose levels in male KM mice treated with TBT chloride by oral gavage once every three days at 0.0005, 0.005, or 0.05 mg/kg bw for a 45 day period. Zuo et al. (2009) did detect changes in leptin and several adipose-secreted proteins (those findings are discussed above).

Some of the early work suggests that rats and mice may be a relatively insensitive model system to look at the effects of organotins on glucose regulation. A study by Matsui et al. (1984) compared the effects of TPT hydroxide on glucose levels in male Japanese white rabbits, golden hamsters, ddY mice, and Hartly guinea pigs treated with a single oral dose of 100 mg/kg and assessed 3 days later. Statistically significant increases in plasma glucose, presumably in non-fasted animals, were only seen in rabbits (3.24-fold) and hamsters (2.93-fold) and there was no suggestion of an effect in the other species tested, including a group of Wistar rats treated with a 200 mg/kg dose. All of the treatment groups lost weight in the 3-day period after treatment and the decreases were statistically significant in all species (-4.4% to -14.4% change from initial weight) except for hamsters (-3.3% change). TPT hydroxide treatment did not cause obvious changes in islet cell histology in any species.

The effects of acute dosing with TPT on glucose homeostasis appear to be transient, at least in hamsters treated with a slightly lower dose than was used in the study by Matsui et al. (1984). Ogino et al. (1996) treated male Golden hamsters with a single gavage dose of 60 mg/kg TPT chloride and conducted IVGTT tests on days 1, 2, 3, 4, and 7 after treatment and observed changes in fasting plasma glucose, basal immunoreactive insulin (IRI), and response in the iv glucose tolerance test (IVGTT; Table 2). All of these effects were transient and there were no differences on any of these endpoints by day 3 post-treatment. No TPT was detected in the pancreas prior to treatment and the highest levels were

Table 2. Summary of glucose and immunoreactive insulin (IRI) findings in the 7-day period after a single gavage dose treatment of Golden hamsters with 60 mg/kg TPT chloride

↑ fasting plasma glucose on days 1 (2.1-fold) and 2 (1.8-fold, but not statistically significant)
↓ basal IRI on days 1 and 2 (46 to 58% of control)
↓ $\Delta\text{IRI}/\Delta\text{PG}$ after IVGTT on day 1 (30% of control)
↑ fasting triglycerides on day 1 (7.56-fold) and 2 (3.16-fold, but not statistically significant)
*No significant treatment effect for any of these endpoints by 3-days after treatment

Data from Ogino et al. (1996)

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found on day 1 (1.43 $\mu\text{g/g}$) and these decreased to 0.64 $\mu\text{g/g}$ on day 3 and to 0.15 $\mu\text{g/g}$ on day 7. A separate experiment by Miura *et al.* (1997) did not detect any significant differences in the proportion of insulin immunoreactive islet cells isolated by collagenase digestion from the pancreas of male Syrian hamsters 2 days after receiving a single oral dose of 60 mg/kg TCT-chloride in sesame oil, or oil alone.

Follow-up studies from this research group suggested that species differences in tissue accumulation of TPT are a major factor for the differences in hyperglycemic response. Ohhira *et al.* (1999) conducted a study to see if more rapid metabolism of TPT via co-treatment with inducers of cytochrome p450s could attenuate the transient hyperglycemic effects seen in hamsters following a single oral dose of 50 mg/kg TPT chloride. The hyperglycemic response seen in animals treated with TPT only at 12-72 hours was less pronounced in animals that were co-treated with TPT and phenobarbital, an inducer of CYP2B, 2C, and 3A. Pancreatic, liver, kidney, and brain levels of TPT in phenobarbital co-treated animals were also lower in the 2 to 3 day period after treatment compared to animals treated with TPT alone. Inducers of CYP1A and 2A (β -naphthoflavone and 3-methylcholanthrene) were less effective with β -naphthoflavone showing no attenuation of TPT-induced hyperglycemic response. In another experiment, ddY mice were pre-treated with [SKF-525A](#),⁷ an inhibitor of several cytochrome P450 enzymes, to see if this would alter the metabolism and toxicity of a single oral dose of 50 mg/kg TPT chloride (Ohhira *et al.* 2000). Liver and pancreatic levels of TPT were higher in mice pre-treated with SKF-525A compared to animals that were not pretreated (liver: 5.94 ± 1.93 vs. 2.18 ± 0.71 $\mu\text{g/g}$ tissue; pancreas: 1.29 ± 0.51 vs. 0.5 ± 0.17 $\mu\text{g/g}$ tissue). Treatment with TPT induced hyperglycemia in mice pre-treated with SKF-525A while the mice without the SKF-525A pre-treatment were not affected. Plasma triglycerides were not altered in TPT-treated mice, regardless of SKF-525A treatment status.

Membrane depolarization leads to the opening of voltage-gated calcium channels and entry of Ca^{+2} ions into β cells to trigger insulin secretion. One hypothesis assessed by the Matsui research group is whether the hyperglycemic effects of TPT chloride could be due to reduced insulin secretion from impaired Ca^{+2} signaling. Support for this concept was provided in several *ex vivo* studies using β -cells isolated from hamsters that were treated with 60 mg/kg TPT chloride. TPT chloride impaired the normal Ca^{+2} response to treatments with (1) 27.8 mM glucose, (2) 100 nM gastric inhibitory polypeptide in the presence of 5.5 mM glucose, and (3) 100 μM acetylcholine in the presence of 5.5 mM glucose (Miura *et al.* 1997). Partial suppression was observed in K^{+} -induced Ca^{2+} rise. The inhibitory effects on Ca^{2+} response due to reduced Ca^{2+} influx occurred after Na^{+} -dependent and Na^{+} -independent depolarization in the islet cells (Miura and Matsui 2001); the Na^{+} -dependent pathways included protein kinase A (PKA)-dependent mechanisms and PKA-independent pathways mediated by cAMP regulated guanine nucleotide exchange factors (Miura and Matsui 2006).

A study by Pace and Tarvin (1982) used TBT as a test compound to look at anion exchange, intracellular pH, and glucose-induced electrical activity in β -cells because TBT is an

⁷ SKF-525A = *o*-phenyl-*o*-propylbenzeneacetic acid 2-[diethylamino]-ethyl ester hydrochloride

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electroneutral Cl:OH exchanger. TBT treatment stimulated the electrical activity of 11.1 mM glucose, causing effects that were considered similar to a decrease in intracellular pH. TBT was also used as a Cl-ionophore in a study by Beauwens et al. (2006). Treatment with 2.5 μ M TBT doubled insulin secretion in the insulin-secreting BRIN-BD11 cell line compared to 1 μ M TBT under isotonic conditions, but reduced the magnitude of insulin release stimulated by incubation in a hypotonic medium. This finding may support the work of Miure et al. (1997) because the insulin secretory response to hypoosmolarity was characterized as a Ca^{+2} -dependent process. Watanabe et al. (2002) conducted experiments to determine whether the TBT-induced effects could be mediated through interactions with K_{ATP} channels, by comparing the response to the [sulfonylurea](#) tolbutamide in control and TBT-treated mice. The effects on insulin secretion from tolbutamide were similar in both groups which led the authors to conclude that TBT was exerting its hyperglycemic effects before the process of membrane depolarization due to interactions of K_{ATP} and sulfonylurea receptors.

1.2.2 Phthalates

Adiposity

Relatively few experimental animal studies have looked at the effects of phthalate treatment on adipocytes, adiposity, or fat/lean tissue composition ([Appendix Table D](#)) although hypolipidemic effects have been reported. In the studies, animals were treated with dose levels of phthalate, mostly DEHP, that are considered high compared to intakes in the general population (2006). These high doses of DEHP cause induction of peroxisomes in the liver in rodents, increased fatty acid oxidation, and reductions in fat tissue mass, through mechanisms that are not fully understood but appear to be primarily mediated through PPAR α (Feige *et al.* 2010; Martinelli *et al.* 2010).

One of the earliest studies reporting these types of effects was a 1978 publication by Sakurai et al (1978). Adult male Wistar rats weighing 100-120 grams were fed a diet containing 0, 1, 2, or 4% DEHP for 1, 2, or 4 weeks (Sakurai *et al.* 1978). **[Doses estimated to be ~500, 1000, and 2000 mg/kg bw/day]** Animals fed the 2 or 4% diets gained less weight than control animals. The weights of the epididymal fat pads were described as “markedly reduced” in the 2 and 4% DEHP groups. Statistically significant decreases in plasma triglycerides (n=5 rats/group) were observed in the 1% and 2% DEHP treatment groups but not in animals fed the 4% DEHP diet. The effects on plasma triglycerides were observed at 1% DEHP after 2 weeks of treatment (54% of control) and at 2% following 1 or 2 weeks of treatment (55 and 49% of control respectively); there was no effect of these dietary treatments in animals treated for 4 weeks. Blood levels of free fatty acid were significantly increased by 2.2 to 2.9-fold in the 2 and 4% dietary groups compared to control levels. Ketone bodies in the blood were higher in these treatment groups and were also increased in the 1% DEHP group after 4 weeks of treatment. Plasma cholesterol levels were not affected by DEHP treatment at any dose level or treatment duration, although it should be noted that cholesterol levels were much lower in control animals in the 4-week

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treatment group compared to the 1 and 2-week treatment groups.⁸ Blood glucose was reduced to 80% of control levels in the 2% and 4% groups and liver glycogen levels were very low, 38% of control levels. Data on liver levels of intermediates in carbohydrate metabolism showed that gluconeogenesis was being inhibited at reaction steps between 3-phosphoglycerate and fructose 1,6-diphosphate. Hepatic fatty acid synthesis was also increased by ~2-fold.

Itsuki-Yoneda et al. (2007, 1317) looked at liver, cardiac, and testicular expression of several enzymes involved in mitochondrial fatty acid oxidation and acetate formation in 6-week old Sprague-Dawley rats receiving a diet containing 2% DEHP for 3 weeks (long-chain acyl-CoA dehydrogenase, 3-ketoacyl-CoA thiolase, acetyl-CoA synthetase 1 and 2, and acetyl-CoA carboxylase). This treatment caused decreased body weight. The overall pattern of results for enzyme expression suggested that DEHP increased fatty acid oxidation and acetate formation in the liver and testes, and increased acetate utilization in the heart.

The reduced adiposity in DEHP treated animals reported by Sakurai et al. (1978) is consistent with other studies. A recent example is a study by Martinelli (2010) reporting that a 21-day dietary treatment with ~1,600 mg/kg bw/d DEHP to adult male Wistar rats caused significant decreases in epididymal adipose (74% of control) and lumbar adipose (67% of control) tissue mass. DEHP also caused decreased body weight to 83% of control body weight, and body weight gain to 66% of control body weight gain, but the effects of reduced adipose mass remained statistically significant when normalized to body weight. Feed intake was unaffected. Adipocytes isolated from the epididymal and lumbar fat samples were smaller in diameter and volume than those isolated from control animals and had less triglyceride content.⁹ The authors also conducted experiments to see if the effects were due to enhanced lipid mobility by looking at basal and noradrenaline-stimulated lipolysis and activity of adipocyte lipoprotein lipase (LPL), an enzyme that catalyzes the hydrolytic cleavage of fatty acids from triglycerides in chylomicrons, very-low-density lipoproteins (VLDLs), and low-density lipoproteins (LDLs). Neither of these parameters was affected by DEHP treatment so it is unclear what biological processes may be causing the decreased fat retention. The authors also called attention to the negative nitrogen balance in animals receiving 2% DEHP reported in earlier studies from this same laboratory (Bernal *et al.* 2002) suggesting an overall phthalate effect on energy expenditure.

The DEHP metabolite, mono-2-ethylhexyl phthalate (MEHP) is capable of activating PPAR γ *in vitro* (Feige *et al.* 2010; Maloney and Waxman 1999) but the expected adipogenic effects of activation of this receptor are not observed *in vivo* when animals are treated with the parent phthalate DEHP. Treated animals typically have decreased body weight and fat mass. These effects appear to be largely mediated via PPAR α agonist activities of DEHP metabolites. Feige et al. (2010) conducted a series of experiments to characterize the underlying molecular

⁸ Plasma cholesterol in control animals at 1, 2, and 4 weeks were 95 \pm 21 mg/100ml, 101 \pm 3 mg/100ml, and 67 \pm 5 mg/100ml

⁹ Effects in isolated adipocytes: \downarrow cell diameter (93% of control), \downarrow cell volume (78% of control), \downarrow TG content (89% of control)

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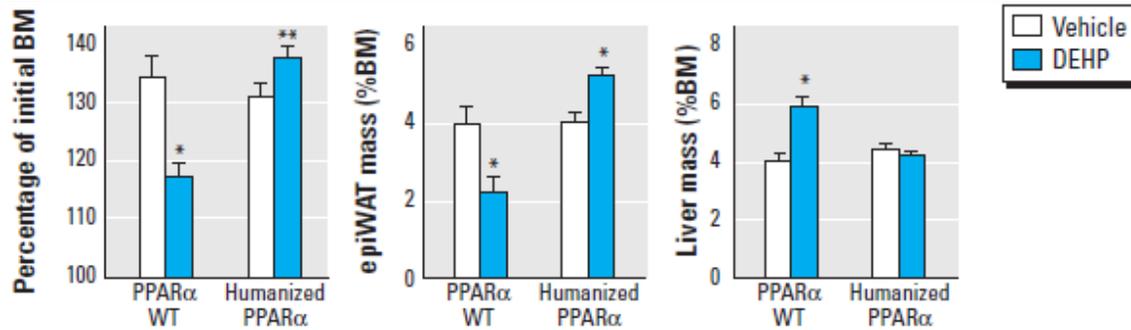
mechanisms of the DEHP metabolic effects. In the initial experiment, wild-type C57Bl6J mice were fed a diet containing 100 or 1,000 mg/kg bw/d DEHP for 13 weeks. The mice treated with 1,000 mg/kg bw/d DEHP weighed less despite having similar daily food consumption as controls. Results from studies using nuclear magnetic resonance (NMR) to measure fat and lean body composition showed no effect on lean mass in this treatment group, but a significant reduction in fat mass (~55% of control). Epididymal white adipose mass as a percent of body mass was also significantly reduced to 63% of controls in the 1,000 mg/kg bw/d treatment group. A follow-up study using 500 mg/kg bw/d DEHP showed that consumption of a high fat diet did not alter the results. The mice still gained less weight despite eating the same amount of food and had significantly decreased fat and epididymal white adipose tissue mass compared to control animals also fed a high fat diet. Lean mass was again unaffected by DEHP treatment. To assess the primary target organs involved in the reduction of fat mass the investigators evaluated the expression of selected genes under the control of PPAR α and/or γ in liver, skeletal muscle and white and brown adipose tissue. The results indicated a primary effect of DEHP on activation of genes under the control of PPAR α in the liver. The investigators then looked at these effects in wild-type and PPAR α KO and PPAR β KO mice treated with 500 mg/kg bw/d DEHP and fed a high fat diet (n=6/group). The effects on body weight and fat mass were present in wild type and PPAR β KO mice, but not PPAR α KO mice demonstrating the importance of PPAR α in regulating the fat loss. The presence of a functional PPAR α receptor was also required for DEHP-induced hepatomegaly, expression of genes involved in hepatic intracellular fatty acid transport and mitochondrial and peroxisome fatty acid oxidation.

The human health implications of a number of environmental chemicals that appear to act through PPAR α in rodents have been a subject of much debate (Klaunig *et al.* 2003). The Feige *et al.* (2010) study also addressed this issue. Using genetically modified mice in which the normal mouse PPAR α gene was replaced with the human gene, the group observed that DEHP treatment did not induce hepatomegaly, or expression of genes involved in fatty acid oxidation and was not effective in preventing weight gain of the “humanized mice” when placed on a high fat diet. In fact, the humanized mice treated with DEHP gained more weight and had an increase in epididymal white adipose mass compared to the wild-type animals (Figure 8). Through a series of glutathione S-transferase (GST) pull down experiments, the Feige group demonstrated that MEHP activation of the human and mouse PPAR α s resulted in subtle differences in the recruitment of co-activator proteins. These differences were less apparent with a “full PPAR α agonist” Wy 14643, potentially accounting for the phenotypic differences observed with the “humanized mice”.

Feige *et al.* (2010) also speculated on the reasons that MEHP was ineffective in stimulating PPAR γ in adipose tissue *in vivo*, despite its often equipotent activation of PPAR γ and α in cellular assays *in vitro* (Table 3). They proposed that MEHP was not entering adipocytes in sufficient concentrations to activate PPAR γ .

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Figure 8. Opposite effects of DEHP in PPAR α WT mice and PPAR α humanized mice



From Feige et al. (2010) an open access article.

A study by Casals-Casas (Casals-Casas *et al.*) looked at the effects of *in utero* DEHP exposure by feeding female C57B16/J mice a diet containing ~200 mg/kg bw/d DEHP before and during pregnancy. At 3 weeks of age there was no difference in F1 offspring with respect to body weight, liver weight, brown and white adipose tissue weights and blood glucose. There was also no difference in growth curves from DEHP treatment when the F1 offspring were placed on a high fat diet from weeks 7 to 19. Similar findings were observed in F1 offspring of untreated dams and DEHP-treated fathers.

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Table 3. Comparison of phthalate activation of PPAR α and PPAR γ

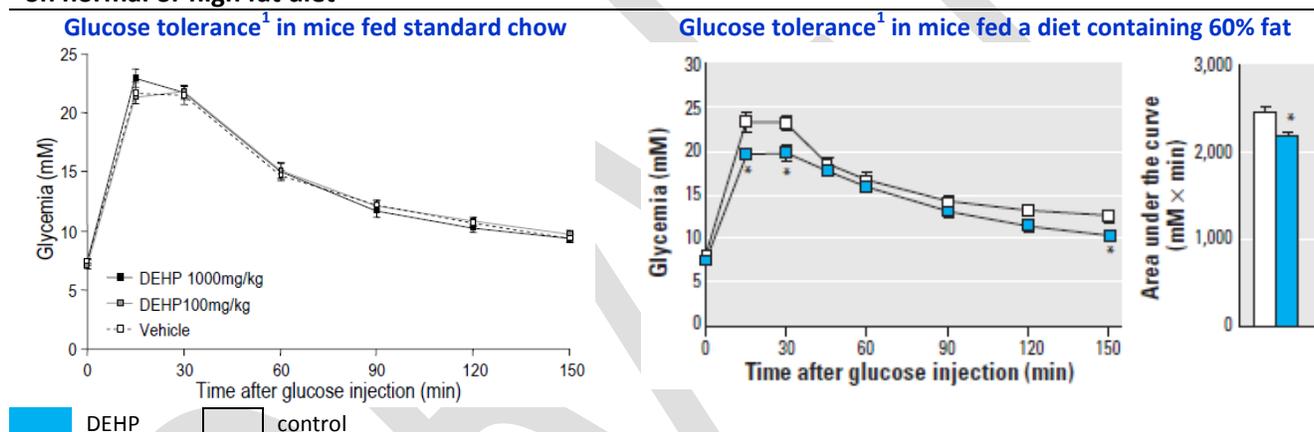
monoester metabolite	reference	LOEC (max fold-induction)				adipogenic activity
		mPPAR α	hPPAR α	mPPAR γ	hPPAR γ	
MEP	(Bility <i>et al.</i> 2004)	--	--	--	10 μ M (3.3-fold)	
	(Hurst and Waxman 2003)	concentration not reported (~2-fold)	--	not reported	not reported	
MButP	(Bility <i>et al.</i> 2004)	100 μ M (3.7-fold)	200 μ M (2.4-fold)	--	--	none at 50 μ M
	(Hurst and Waxman 2003)	10 μ M (~3-fold)	--	300 μ M (~2-fold)	not reported	\uparrow 100 and 300 μ M
MEHP	(Bility <i>et al.</i> 2004)	10 μ M (11.1-fold)	30 μ M (4.8-fold)	30 μ M (2.5-fold)	10 μ M (5.5-fold)	strong induction at 50 μ M
	(Hurst and Waxman 2003)	0.5 μ M (~2.5-fold)	4 μ M (~2.3-fold)	3 μ M (~3.7-fold)	5 μ M (~2.8-fold)	strong induction at 50 μ M
	(Maloney and Waxman 1999)	5 μ M (4.2-fold)	5 μ M (3.1-fold)	5 μ M (3.7-fold)	1 μ M (3.2-fold)	
	(Feige <i>et al.</i> 2007)	~3.2 μ M* (~2.3-fold max)		mPPAR γ 1 ~3.2 μ M* (~2-fold max)	hPPAR γ 1 ~10 μ M* (~2.5-fold max) hPPAR γ 2 ~3.2 μ M* (~2.3-fold max)	significant induction
	Blumberg, personal communication			<u>weak activation</u>	<u>weak activation</u>	slight \uparrow in isolated mouse stem cells; no effect in isolated human stem cells
DEHP	(Hurst and Waxman 2003)	--	--	not reported	not reported	
	(Maloney and Waxman 1999)	--	--	--	--	
	Blumberg, personal communication					strong dose-dependent \uparrow in isolated human stem cells, slight dose-dependent \uparrow in isolated mouse stem cells
MBzP	(Hurst and Waxman 2003)	10 μ M (~4.6-fold)	30 μ M (~2.3-fold)	10 μ M (~4-fold)	30 μ M (~4-fold)	\uparrow 100 and 300 μ M

*LOEC not statistically analyzed

Glucose homeostasis and pancreatic effects

The effects of DEHP on glucose tolerance are mixed. In the Feige et al. (2010) study described above, plasma glucose levels were not affected by DEHP treatment of 100 or 1,000 mg/kg bw/d in either fed or fasted C57Bl6 mice and glucose tolerance was not impacted during an ip glucose tolerance test (IPGTT) where animals were fasted for 4 hours prior to injection with 2 g glucose/kg body weight¹⁰ (Figure 9). Insulin levels were 50% lower in animals treated with 1,000 mg/kg bw/d DEHP compared to control. Glucose tolerance was enhanced in mice treated with 500 mg/kg bw/d while consuming a high fat diet. The area under the curve, or AUC_(0-150 minutes) was ~2,500 and ~2,200 mM x min in controls and treated mice, respectively. These findings are interpreted as enhanced glucose tolerance, potentially a result of the leaner phenotype in the DEHP treated animals rather than a direct effect of the compound.

Figure 9. Glucose tolerance in C57Bl6 mice treated with 100, 500, or 1000 mg/kg bw/d DEHP for 13-weeks on normal or high fat diet



¹Animals were fasted for 4 hours prior to injection with 2 g glucose/kg body weight
From Feige et al. (2010) an open access article.

Two other studies also conducted glucose tolerance tests and reported impaired glucose tolerance (Martinelli *et al.* 2006; Mushtaq *et al.* 1980). Mushtaq *et al.* (1980) reported impaired glucose tolerance in adult male albino rats treated with three ip injections of 5 ml/kg-d during a 10 day period [the actual DEHP dose per kg bw cannot be calculated in this paper, only the volume of the injection was presented]. On day 22 of the experiment, 12 days after the last DEHP treatment, animals were fasted for 16 hours and then challenged with an oral dose of 2 g glucose/kg body weight. Blood glucose was higher compared to controls at 45, 90, and 135 minutes after the glucose load. Martinelli *et al.* (2006) also saw impaired glucose tolerance in adult male Wistar rats that were fed a diet containing 2% DEHP for 21 days [~1000 mg/kg bw/day]. At the end of the treatment period, the animals were fasted for 15 – 17 hours and

¹⁰ IPGTT: ↑Plasma glucose at 15 and 30 minutes (1.24-fold); ↓AUC (97.3% of control)

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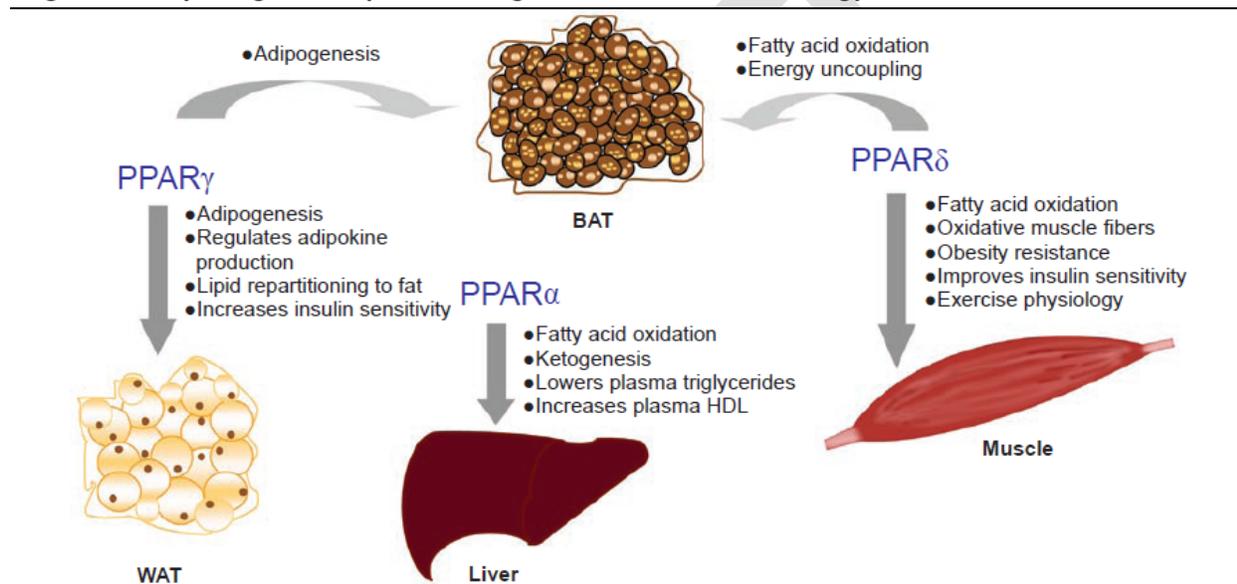
then challenged with an oral glucose dose of 2 g/kg body weight. Plasma glucose levels were higher at the start of the study as well as after 90 minutes. The $AUC_{0-180 \text{ minutes}}$ was significantly higher in DEHP-treated rats compared to controls (35 ± 2.4 versus $27.3 \pm 2.4 \text{ mg/dl} \times \text{min} \times 10^3$). Effects on body weight were not reported in Mushtaq et al. (1980), but the DEHP dosed rats in Martinelli et al. (2006) gained less weight than control animals during the treatment period despite having similar daily food energy intake. Sakurai et al. (1978) reported decreased blood glucose (~80% of control) in non-fasted male Wistar rats fed a diet containing 2% DEHP [**~1000 mg/kg bw/day**] for weeks. An *in vitro* study by Rengarajan (2007) in Chang liver cells reported that a 24hour incubation with high concentrations of DEHP (200 and 400 μM) decreased insulin receptor concentration and glucose oxidation; cell viability was not affected.

Fewer studies have reported findings for other phthalates. Boberg et al. (2008) exposed timed pregnant Wistar rats to 600 mg/kg/day di-isobutyl phthalate (DiBP), by oral gavage on GD 7 through GD 19 or 21, at which time the animals were sacrificed and fetal blood collected for hormone analysis. Among the panel of hormones assayed, both insulin and leptin levels were significantly lower in the fetuses from dams receiving DiBP. These findings were part of a larger effort to examine a number of different chemicals and drugs that are known to interact with PPARs as well as affecting steroidogenesis, particularly testosterone synthesis.

1.3 Environmental Chemicals and PPAR, RXR, and RAR activity

Peroxisome proliferating receptors (PPARs) are well-established as important regulators of adipogenesis, insulin sensitivity, fatty acid oxidation, lipid regulation and other aspects of metabolic homeostasis (Figure 10). In toxicology, the historical focus has been on the role of PPAR α agonists in liver toxicity and carcinogenesis in rodents and the relevance of this mechanism of action to humans (Klaunig *et al.* 2003). Less explored are interactions with PPAR γ and other PPAR receptor subtypes on energy regulation.

Figure 10. Physiological and pharmacological roles of PPARs in energy metabolism



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Of the PPAR receptors, environmental chemicals with PPAR γ agonist activity are more likely to be linked to adipogenic effects. PPAR γ agonists enhance insulin sensitivity and some are used as oral medications to control diabetes. Only two PPAR γ agonist medications are currently available for daily use (pioglitazone and rosiglitazone), and others have been withdrawn from the market or been labeled with safety alerts due to concern for adverse cardiac or liver events (Kaul *et al.* 2010; Rizos *et al.* 2009). PPAR γ agonists are also adipogenic agents and are commonly used as positive controls in experiment studies (Figure 11). Recent work by Choi *et al.* (2010) suggests that binding affinity to PPAR γ is not likely to be predictive of the adipogenic versus the anti-diabetogenic effects of a drug. The therapeutic effects of PPAR γ agonists for diabetes are linked to their ability to prevent a kinase (Cdk5) from phosphorylating PPAR γ , an activity stimulated by high fat diets and causing increased production of insulin-resistance adipokines. Strong and weak PPAR γ agonists were equally effective in blocking PPAR γ phosphorylation and were equally effective in restoring a non-diabetic pattern of gene expression (Choi and Kim 2010). Stimulation of PPAR γ may actually be the reason for the

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reported side effects that include weight gain with long term use. The overall implication is that binding without receptor activation may be beneficial for diabetes, but activation of the PPAR γ receptor does appear to be required for the adipogenic effects of chemicals that bind to the receptor. The potent effects of organotins on adipogenesis are attributed to dual agonistic activity for PPAR γ and RXR (Hiromori *et al.* 2009; Inadera and Shimomura 2005; Kanayama *et al.* 2005; Kirchner *et al.* 2010; le Maire *et al.* 2009; Nakanishi *et al.* 2005; Nishikawa *et al.* 2004), although there is some debate on whether these actions are primarily due to interactions with PPAR γ (Kirchner *et al.* 2010) or RXR (le Maire *et al.* 2009).

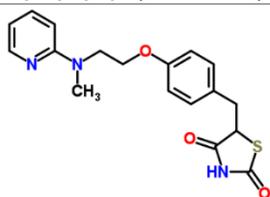
Figure 11. Thiazolidinediones (TZDs) commonly used as positive controls in experimental studies

Rosiglitazone

(Avandia)

122320-73-4

C₁₈H₁₉N₃O₃S (MW 357.4)

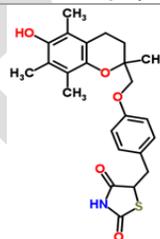


Troglitazone

(Rezulin, Resulin or Romozin)

97322-87-7

C₂₄H₂₇NO₅S (MW 441.5)



Anti-diabetogenic drug placed under selling restrictions in the US in August 2007 due to an increased risk of cardiovascular events (Kaul *et al.* 2010).

Anti-diabetogenic drug withdrawn from the US market in 2000 due to increased incidence adverse liver effects (Rizos *et al.* 2009).

Effects of TZDs (reviewed in Kahn and McGraw, 2010):

Beneficial effects:

Adipose tissue

Increased adipocyte differentiation but no increase in cell size
Improved adipokine profile

Macrophages

Decreased inflammatory changes

Blood vessels

Improved endothelial function
Decreased atherosclerosis

Liver

Decreased glucose production
Decreased steatosis

Muscle

Increased insulin sensitivity

Adverse effects

Congestive heart failure
Fluid retention
Weight gain
Bone fractures
Edema

Variable effects

Plasma lipid profiles

Bone marrow is a source of MSC capable of differentiating into osteoblasts and adipocytes, and data suggest that PPAR γ is a crucial mediator of differentiation in this organ. A prototypic TZD, rosiglitazone, activates PPAR γ and induces adipocyte differentiation in bone marrow stromal cells (Gimble *et al.* 1996). Exposure of rodents to rosiglitazone and pioglitazone results in significant bone loss with a concomitant increase in fat content and expression of adipocyte specific markers, similar to aging bone (Lazarenko *et al.* 2007; Rzonca *et al.* 2004; Syversen *et al.*

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2009). Likewise, exposure of long-term human BM cultures to TBT generates adipocytes (Carfi *et al.* 2008), and TBT suppresses the osteogenic capacity of rat calvarial osteoblasts and human and mouse adipose-derived stromal stem cells (Adeeko *et al.* 2003; Kirchner *et al.* 2010; Tsukamoto *et al.* 2004). Osteoporosis has been linked to “obesity of the bone” (Rosen and Bouxsein 2006), and treatment with PPAR γ agonists can exacerbate osteoporotic pathology. Human treatment with TZDs is associated with an increased risk of fracture and adipogenesis in bone (Loke *et al.* 2009; McDonough *et al.* 2008).

Peer-reviewed literature

Two studies in the peer-reviewed literature have surveyed environmental chemicals for interactions with the different isoforms of PPAR (Maloney and Waxman 1999; Takeuchi *et al.* 2006). Takeuchi *et al.* (2006) characterized mouse PPAR α and PPAR γ agonistic activities for 200 pesticides¹¹ by *in vitro* reporter gene assays using CV-1 monkey kidney cells. Only three of the compounds were considered to have PPAR α -mediated transcriptional activities in a dose-dependent manner: diclofop-methyl (a diphenyl ether), pyrethrins (a pyrethroid) and imazalil (an imidazole) (Table 4). These 3 compounds did not display mRXR α -mediated transcriptional activity in a CV-1 cell-based reporter gene assay. None of the 200 pesticides showed PPAR γ agonistic activity at concentrations $\leq 10^{-5}$ M¹². Diclofop-methyl, pyrethrins and imazalil were then tested *in vivo* for their ability to increase gene expression of PPAR α -inducible cytochrome P450 4As (CYP4As) in the liver of female C57BL/6 mice 18 hours after a single ip injection treatment. Significant induction of CYP4A10 and CYP4A14 mRNA levels were seen in mice treated with diclofop-methyl (3 and 30 mg/kg) and pyrethrins (300 mg/kg), whereas imazalil induced almost no gene expression when tested at 100 and 300 mg/kg. Diclofop-methyl was considered especially potent, causing a magnitude of response similar to the positive control for PPAR α agonist activity, WY-14643.

Table 4. Relative effective concentration for PPAR α agonistic activity

Compound	REC ₂₀ (μ M)
WY-14643	0.49
diclofop-methyl	3.7
pyrethrins	1.8
imazalil	2.5

REC₂₀ (μ M) is the concentration of the test compound showing 20% of the agonistic activity of 10 μ M WY-14643
From Takeuchi *et al.* (2006)

A smaller set of compounds were assessed by Maloney *et al.* (1999) for mouse PPAR α and PPAR γ agonistic activities (Appendix Table F). PFOA and DEHP were the most potent PPAR α agonists and MEHP was the only compound to act as a PPAR γ agonist for both mouse and human PPAR γ .

¹¹ 29 organochlorines, 11 diphenyl ethers, 56 organophosphorus pesticides, 12 pyrethroids, 22 carbamates, 11 acid amides, 7 triazines, 8 ureas and 44 others

¹² A concentration of 10^{-5} , or 10 μ M, might be too low to identify activity based on ToxCast data.

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Recently Sargis et al. (2010) reported studies looking at the ability of 13 compounds (alkyl tin compounds, insecticides, fungicides, polychlorinated biphenyls, plasticizers)¹³ to promote protein expression and lipid accumulation in preadipocytes in culture through stimulation of the glucocorticoid receptor or activation of a PPAR response element. In the presence of a differentiation cocktail, four test materials were found to stimulate the preadipocytes at 100 nM through activation of glucocorticoid receptor independent of PPAR γ , bisphenol A, dicyclohexyl phthalate, endrin and tolylfluaniid.

Activities of PFOS, PFOA and other perfluorinated chemicals (PFCs, also called perfluoroalkyl acids or PFAAs)¹⁴ have also been characterized with respect to PPAR activities (Takacs and Abbott 2007; Wolf *et al.* 2008). Neither PFOS nor PFOA were considered active for mouse or human PPAR γ in transactivation assays, but both were active for PPAR α (PFOA > PFOS) and PPAR δ (Table 5). The PFCs of longer chain length were generally most active, as were the carboxylates (PFBA, PFHxA, PFOA, PFNA, PFDA) compared to the sulfonates (PFBS, PHxS, and PFOS) (Table 6).

Table 5. Summary for the LOEC for PFOA and PFOS transactivation of Mouse and Human PPAR α , β/δ , and γ

PPAR isoform	PFAA	mouse LOEC (normalized RLU)	human LOEC (normalized RLU)
α	PFOA	10 μ M (1.7 RLU)	30 μ M (1.7 RLU)
	PFOS	120 μ M (1.5 RLU)	NA
β/δ	PFOA	40 μ M (5.0 RLU)	NA
	PFOS	20 μ M (1.7RLU)	NA
γ	PFOA	NA	NA
	PFOS	NA	NA

RLU – relative light units

LOEC = lowest concentration (μ M) at which there was a significant difference compared to negative control water (ANOVA, p,0.05)

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¹³ tributyltin, TPT, tolylfluaniid, aldicarb, endrin, 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene, Aroclor 1254, PCB 77, PCB 126, PCB 154, benzylbutyl phthalate, dicyclohexyl phthalate and bisphenol A

¹⁴ PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonate; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonate; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonate; PFNA = perfluorononanoic acid; and PFDA = perfluorodecanoic acid

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Table 6. Summary of PFAA transactivation of mouse and human PPAR α in transiently transfected COS-1 cells

PFAA	Species	Dose range tested (μ M)	LOEC, μ M
PFBA (C4)	mouse	5-100	40
	human	5-100	40
PFHxA (C6)	mouse	5-100	20
	human	5-100	10
PFOA (C8)	mouse	0.5-100	1
	human	0.5-100	10
PFNA (C9)	mouse	0.5-100	5
	human	0.5-100	5
PFDA (C9)	mouse	5-100	5
	human	5-100	>100
PFBS (C4)	mouse	1-250	150
	human	1-250	30
PFHxS (C6)	mouse	5-100	20
	human	5-100	10
PFOS (C8)	mouse	1-250	90
	human	1-250	30

 = LOEC \leq 10 μ M

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ToxCast™

One organotin, several phthalates, PFOS and PFOA were included in Phase 1 of ToxCast™, the EPA's contribution to the high throughput screening (HTS) activities of Tox21¹⁵. Phase I of ToxCast™ evaluated 309 chemicals, primarily pesticide active ingredients, in 467 HTS assays that make use of 9 technology platforms. These platforms included both cell-free (biochemical) and cell-based measures in multiple human primary cells, human or rodent cell lines, and rat primary hepatocytes (Judson *et al.* 2010; Reif *et al.* 2010). Half-maximal activity concentrations (AC₅₀) are determined for each assay. A wide spectrum of biological targets or effects is covered, including cytotoxicity, cell growth, genotoxicity, enzymatic activity, receptor binding, ion channels, transcription factor activity and downstream consequences, gene induction, and high-content imaging of cells (Judson *et al.* 2010). Detailed information about each assay included in ToxCast™ is available from the ToxCast™ web site (<http://www.epa.gov/ncct/ToxCast™>).

The organotin triphenyltin hydroxide (fentin), was more active in ToxCast™ compared to any of the phthalates or perfluorinated chemicals that were tested, including interactions with PPAR γ

¹⁵ Tox21 is a collaborative program between the EPA, NIEHS/NTP, NIH/NCGC, and FDA designed to research, develop, validate and translate innovative chemical testing methods that characterize toxicity pathways. Information on ToxCast and Tox21 is available at <http://epa.gov/ncct/Tox21/> as well as overviews by Judson et al. (2010) and Shukla et al. (2010).

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(AC_{50} = 0.01 to 0.54 μ M), PPAR α (AC_{50} = 0.01 μ M), PPAR δ (AC_{50} = 0.01 μ M), and RXR β (AC_{50} = 0.01 μ M) (Table 7). In addition, fentin was active in assays for other receptor systems important in regulating glucose homeostasis, pancreatic function, and feeding behavior at concentrations below 10 μ M, in particular dopamine, β -adrenergic, serotonin, and muscarinic cholinergic receptors (interactions at $>10\mu$ M are presented in Appendix Table G). Whether or not the ToxCast™ data helps reconcile the different *in vivo* activities of these chemicals remains to be seen, but it indicates that focusing only on PPAR activities is likely an overly simplistic approach.

A number of other receptor systems discussed in this chapter are also included in ToxCast™ (Table 8). The screening output for Phase 1 of ToxCast™ is presented in Appendix H. Eight compounds were considered active in four or more assays: bromoxynil (5 assays), imazalil, an imidazole (5 hits), quintozone (5 hits), d-cis,trans-Allethrin (4 hits), fludioxonil (4 assays), flusilazole (4 assays), PFOS (4 assays), and resmethrin (4 assays).

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Table 7. ToxCast™ HTS data for the organotin fentin, several phthalates, PFOS and PFOA (limited to activities at <10μM)

Gene Symbol	Official Full Name	Fentin	PFOS	PFOA	DBP	MBP	DEHP	MEHP	DMP	MMP
ABCB11	ATP-binding cassette, sub-family B (MDR/TAP), member 11	5.21								
ABCG2	ATP-binding cassette, sub-family G (WHITE), member 2	0.05								
ADORA2A	adenosine A2a receptor	1.04	6.90							
ADRA2A	adrenergic, alpha-2A-, receptor	5.79								
ADRA2C	adrenergic, alpha-2C-, receptor	0.43	8.45							
ADRB1	adrenergic, beta-1-, receptor	0.15								
BACE1	beta-site APP-cleaving enzyme 1	25.7	0.53	5.01						
CHRM3	cholinergic receptor, muscarinic 3	6.1								
CHRM5	cholinergic receptor, muscarinic 5	0.7								
COL3A1	collagen, type III, alpha 1		4.44							
COL3A1	collagen, type III, alpha 1				1.48					
CSF1	colony stimulating factor 1 (macrophage)						1.48			
CSF1	colony stimulating factor 1 (macrophage)		1.48							
CSF1	colony stimulating factor 1 (macrophage)				1.48					
CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)		1.48							
CXCL9	chemokine (C-X-C motif) ligand 9						4.44			
CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1	9.19								
CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1				5.92					
CYP1A2	cytochrome P450, family 1, subfamily A, polypeptide 2	5.59			5.88					
CYP1A2	cytochrome P450, family 1, subfamily A, polypeptide 2	7.03								
Cyp2B6	cytochrome P450, family 2, subfamily B, polypeptide 6	3.44			4.95		3.97			
Cyp2B6	cytochrome P450, family 2, subfamily B, polypeptide 6						5.88	9.51		
Cyp2B6	cytochrome P450, family 2, subfamily B, polypeptide 6			6.23			1.38			
Cyp2c11	cytochrome P450, subfamily 2, polypeptide 11		0.06							
CYP2C18	cytochrome P450, family 2, subfamily C, polypeptide 18		1.10							
CYP2C19	cytochrome P450, family 2, subfamily C, polypeptide 19	4.64								

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Table 7. ToxCast™ HTS data for the organotin fentin, several phthalates, PFOS and PFOA (limited to activities at <10µM)

Gene Symbol	Official Full Name	Fentin	PFOS	PFOA	DBP	MBP	DEHP	MEHP	DMP	MMP
CYP2C8	cytochrome P450, family 2, subfamily C, polypeptide 8	6.36								
CYP2C9	cytochrome P450, family 2, subfamily C, polypeptide 9									0.35
CYP2C9	cytochrome P450, family 2, subfamily C, polypeptide 9		0.08					8.85		
CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4	4						5.77		
CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4			2.37	4.17					
CYP4F12	similar to cytochrome P450, family 4, subfamily F, polypeptide 12	7.99								
DRD1	dopamine receptor D1	0.17								
DRD2	dopamine receptor D2	0.16								
DRD2	dopamine receptor D2	0.73								
DUSP3	dual specificity phosphatase 3	0.24								
EGFR	epidermal growth factor receptor					1.48				
Grm1	glutamate receptor, metabotropic 1	4.51								
GSTA2	glutathione S-transferase alpha 2	5.65								
HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	6.24		0.25						0.05
HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)			6.5						
HTR5A	5-hydroxytryptamine (serotonin) receptor 5A	1.84								
HTR6	5-hydroxytryptamine (serotonin) receptor 6	7								
HTR7	5-hydroxytryptamine (serotonin) receptor 7	0.2								
IL1A	interleukin 1, alpha				4.44					
IL9	interleukin 9		4.44							
MMP1	matrix metalloproteinase 1 (interstitial collagenase)									4.44
MMP9	matrix metalloproteinase 9		4.44				1.48			
NFE2L2	nuclear factor (erythroid-derived 2)-like 2	0.02								
NR1H2	Pregnane X receptor	1.14								
NR3C1	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	0.54								
NR4A2	NGFI-B/nur77 beta-type transcription factor homolog	0.02								

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Table 7. ToxCast™ HTS data for the organotin fentin, several phthalates, PFOS and PFOA (limited to activities at <10μM)

Gene Symbol	Official Full Name	Fentin	PFOS	PFOA	DBP	MBP	DEHP	MEHP	DMP	MMP
OPRL1	opiate receptor-like 1	0.18								
Oprl1	opiate receptor-like 1		2.28							
PLAU	plasminogen activator, urokinase		1.48			1.48				
PLAUR	plasminogen activator, urokinase receptor				1.48					
PLAUR	plasminogen activator, urokinase receptor		1.48			1.48				
PPARA	peroxisome proliferator-activated receptor alpha	0.01								
PPARA	peroxisome proliferator-activated receptor alpha							6.46		
PPARD	peroxisome proliferator-activated receptor delta	0.01								
PPARG	peroxisome proliferator-activated receptor gamma	0.01								
PPARG	peroxisome proliferator-activated receptor gamma	0.02								
PPARG	peroxisome proliferator-activated receptor gamma	0.3								
PPP2CA	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform		5.97							
PTPRF	protein tyrosine phosphatase, receptor type, F		9.32							
RARA	retinoic acid receptor, alpha		3.90							
RXRB	retinoid X receptor, beta	0.01								
SERPINE3	serpin peptidase inhibitor, clade E member 3									4.44
SLC6A2	solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2									
SLCO1B1	solute carrier organic anion transporter family, member 1B1							0.01		
SLCO1B1	solute carrier organic anion transporter family, member 1B1							0.02		
SULT2A1	sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1	1.33					0.48	10.45		
TEK	TEK tyrosine kinase, endothelial		4.43							
TGFB1	transforming growth factor, beta 1							4.44		
TSPO	translocator protein (18kDa)						8.93			

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Table 8. ToxCast™ assays for PPARs, RXRs, RARs, and RORs

Receptor	Assay ¹	Assay Technology	Cell Line	Reference Compound(s)	Number of Actives (309 tested)
PPAR α	ATG PPARα TRANS	reporter gene assay, DNA sequencer	human, HepG2 cell line	rosiglitazone	9
	NCGC PPARα Agonist	reporter gene assay, agonist	human, HEK293T cell line	L65041	8
	NVS NR hPPARα	competitive binding, FRET	human, ligand-binding	GW7647	4
PPAR δ	ATG PPARδ TRANS	reporter gene assay, DNA sequencer	human, HepG2 cell line	rosiglitazone	1
	NCGC PPARδ Agonist	reporter gene assay, agonist	human, HEK293T cell line	L65041	0
PPAR γ	ATG PPARγ TRANS	reporter gene assay, DNA sequencer	human, HepG2 cell line	rosiglitazone	146
	NCGC PPARγ Agonist	reporter gene assay, agonist	human, HEK293 cell line	rosiglitazone	4
	NVS NR hPPARγ	competitive binding, fluorescence polarization	human, recombinant	ciglitazone	10
RXR α	ATG RXRα TRANS	reporter gene assay, DNA sequencer	human, HepG2 cell line	9-cis retinoic acid	0
	NCGC RXRα Agonist	reporter gene assay, agonist	human, HEK293T cell line	9-cis retinoic acid	6
RXR β	ATG RXRβ TRANS	reporter gene assay, DNA sequencer	human, HepG2 cell line	9-cis retinoic acid	8
RAR α	ATG RARα TRANS	reporter gene assay, DNA sequencer	human, HepG2 cell line	retinoic acid (all trans and 9-cis)	49
	NVS NR hRAR	receptor activation, FRET	human, recombinant	RO415253	5
RAR β	ATG RARβ TRANS	reporter gene assay, DNA sequencer	human, HepG2 cell line	retinoic acid (all trans and 9-cis)	5
RAR γ	ATG RARγ TRANS	reporter gene assay, DNA sequencer	human, HepG2 cell line	retinoic acid (all trans and 9-cis)	4
RORA	ATG RORE CIS	reporter gene assay, DNA sequencer	human, HepG2 cell line	orphan	36
RORB	ATG RORβ TRANS	reporter gene assay, DNA sequencer	human, HepG2 cell line	orphan	1
RORC	ATG RORγ TRANS	reporter gene assay, DNA sequencer	human, HepG2 cell line	orphan	1

Appendices

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1.4 Appendix Tables

Appendix Table A. Association between urinary phthalate metabolites and indicators of obesity or insulin resistance.					
Urine Metabolite	Waist Circumference (cm)	Body Mass Index, BMI (kg/m ²)	Waist Height Ratio	HOMA (ln)	Reference
MEP					
>20 years	NHANES 1999-2002, adjβ(SE) n=1,292 ♂ 0.66 (0.31), p=0.041*			NHANES 1999-2002, adjβ(SE) n=622 ♂ 0.044 (0.021), p=0.045	Stahlhut et al. (2007) ¹
	NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend		NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend		Hatch et al. (2008) ²
6-11 years (n= 164♂; 163♀)	♂-0.67 (-4.42, 3.09), p=0.99 ♀1.05 (-3.30, 5.40), p=0.61	♂-0.02 (-1.49, 1.46), p=0.65 ♀0.30 (-1.22, 1.81), p=0.66			
12-19 years (n= 330♂; 340♀)	♂-1.20 (-5.14, 2.74), p=0.64 ♀4.11 (0.37, 7.86), p=0.02*	♂-0.13 (-1.63, 1.37), p=0.89 ♀1.74 (-0.02, 3.49), p=0.03*			
20-59 years (n= 447♂; 380♀)	♂2.19 (-0.51, 4.90), p=0.11 ♀2.07 (-0.72, 4.85), p=0.10	♂0.82 (-0.15, 1.79), p=0.11 ♀0.92 (-0.51, 2.36), p=0.14			
60-80 years (n= 182♂; 172♀)	♂1.68 (-1.75, 5.10), p=0.21 ♀-0.22 (-3.49, 3.04), p=0.82	♂1.05 (-0.10, 2.21), p=0.03* ♀-0.21 (-1.73, 1.32), p=0.64			
adult Mexican non-diabetic (n=182)	Spearman correlation ♀ -0.0361, NS	Spearman correlation ♀ -0.0473, NS	Spearman correlation ♀ -0.0414, NS		Svensson et al. (2010)
MBP					
>20 years	NHANES 1999-2002, adjβ(SE) n=1,292 ♂ 0.79 (0.47), p=0.106			NHANES 1999-2002, adjβ(SE) n=622 ♂ 0.043 (0.023), p=0.081	Stahlhut et al. (2007) ¹
	NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend		NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend		Hatch et al. (2008) ²
6-11 years (n= 163♂; 163♀)	♂p= 1.25 (-1.91, 4.40), p=0.86 ♀0.37 (-2.67, 3.40), p=0.84	♂0.80 (-0.42, 2.03), p=0.56 ♀0.07 (-1.12, 1.27), p=0.55			
12-19 years (n= 330♂; 342♀)	♂-1.47 (-5.41, 2.48), p=0.31 ♀-0.47 (-4.71, 3.77), p=0.31	♂-0.87 (-2.54, 0.79), p=0.20 ♀-0.17 (-2.24, 1.90), p=0.20			
20-59 years (n= 445♂; 381♀)	♂2.91 (0.22, 5.60), p=0.01* ♀-2.60 (-6.15, 0.95), p=0.24	♂0.65 (-0.39, 1.69), p=0.11 ♀-1.43 (-3.37, 0.52), p=0.29			
60-80 years (n= 182♂; 174♀)	♂-2.60 (-6.05, 0.85), p=0.08 ♀-5.67 (-9.31, -2.03), p=0.01*	♂-1.12 (-2.49, 0.24), p=0.04* ♀-2.69 (-4.54, -0.84), p=0.01*			
adult Mexican non-	Spearman correlation	Spearman correlation	Spearman correlation		Svensson et al. (2010)

Appendices

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Appendix Table A. Association between urinary phthalate metabolites and indicators of obesity or insulin resistance.

Urine Metabolite	Waist Circumference (cm)	Body Mass Index, BMI (kg/m ²)	Waist Height Ratio	HOMA (ln)	Reference
diabetic (n=182)	♀ -0.0478, NS	♀ 0.0249, NS	♀ -0.0020, NS		
MBzP					
>20 years	NHANES 1999-2002, adjβ(SE) n=1,292 ♂ 1.09 (0.36), p=0.005*		NHANES 1999-2002, adjβ(SE) n=622 ♂ 0.061 (0.022), p=0.009		Stahlhut et al. (2007) ¹
	NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend		NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend		Hatch et al. (2008) ²
6-11 years (n= 164♂; 163♀)	♂0.55 (-3.31, 4.40), p=0.85 ♀-0.50 (-3.66, 2.66), p=0.65	♂-0.13 (-1.53, 1.28), p=0.80 ♀-0.18 (-1.43, 1.08), p=0.80			
12-19 years (n= 332♂; 340♀)	♂3.10 (-0.67, 6.88), p=0.15 ♀1.46 (-3.06, 5.98), p=0.74	♂0.84 (-0.47, 2.15), p=0.30 ♀0.84 (-0.97, 2.65), p=0.59			
20-59 years (n= 446♂; 379♀)	♂ 6.63 (3.42, 9.84), p<0.0001* ♀3.18 (-0.90, 7.26), p=0.29	♂2.35 (1.04, 3.65), p=0.0002* ♀0.82 (-1.26, 2.90), p=0.62			
60-80 years (n= 182♂; 176♀)	♂-3.18 (-7.64, 1.28), p=0.09 ♀-2.41 (-6.65, 1.84), p=0.24	♂-1.59 (-3.43, 0.24), p=0.06 ♀-0.73 (-2.67, 1.22), p=0.49			
adult Mexican non-diabetic (n=182)	Spearman correlation ♀ -0.0063, NS	Spearman correlation ♀ 0.0059, NS	Spearman correlation ♀ 0.0883, NS		Svensson et al. (2010)
MEHP					
>20 years	NHANES 1999-2002, adjβ(SE) n=1,292 ♂ 0.53 (0.42), p=0.217		NHANES 1999-2002, adjβ(SE) n=622 ♂ 0.016 (0.024), p=0.526		Stahlhut et al. (2007) ¹
	NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend		NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend		Hatch et al. (2008) ²
6-11 years (n= 163♂; 163♀)	♂-0.20 (-2.98, 2.57), p=0.96 ♀-2.51 (-6.52, 1.49), p=0.33	♂-0.22 (-1.32, 0.89), p=0.76 ♀-0.90 (-2.51, 0.71), p=0.45			
12-19 years (n= 331♂; 345♀)	♂-1.39 (-5.15, 2.37), p=0.44 ♀-2.18 (-4.99, 0.63), p=0.10	♂-0.50 (-1.95, 0.94), p=0.46 ♀-1.51 (-2.81, -0.21), p=0.02*			
20-59 years (n= 244♂; 387♀)	♂0.91 (-1.43, 3.24), p=0.44 ♀-2.17 (-5.99, 1.65), p=0.08	♂0.44 (-0.63, 1.52), p=0.35 ♀-1.68 (-3.57, 0.21), p=0.02*			
60-80 years (n= 196♂; 199♀)	♂-2.42 (-5.76, 0.93), p=0.16 ♀-4.15 (-7.48, -0.81), p=0.05*	♂-1.16 (-2.60, 0.28), p=0.08 ♀-2.07 (-3.42, -0.73), p=0.01*			
adult Mexican non-diabetic (n=182)	Spearman correlation ♀ -0.0236, NS	Spearman correlation ♀ -0.0668, NS	Spearman correlation ♀ 0.0336, NS		Svensson et al. (2010)
MEHHP					
>20 years	NHANES 1999-2002, adjβ(SE) n=1,292		NHANES 1999-2002, adjβ(SE) n=622		Stahlhut et al. (2007) ¹

Appendices

(version updated January 5, 2011)

Appendix Table A. Association between urinary phthalate metabolites and indicators of obesity or insulin resistance.					
Urine Metabolite	Waist Circumference (cm)	Body Mass Index, BMI (kg/m ²)	Waist Height Ratio	HOMA (ln)	Reference
	♂ 1.65 (0.50), p=0.005*			♂ 0.038 (0.023), p=0.126	
	NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend	NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend			Hatch et al. (2008) ²
6-11 years (n= 96♂; 86♀)	♂ 1.27 (-2.43, 4.96), p=0.40 ♀ 1.83 (-3.48, 7.13), p=0.42	♂ 0.42 (-1.09, 1.92), p=0.57 ♀ 0.54 (-1.50, 2.57), p=0.40			
12-19 years (n= 161♂; 170♀)	♂ 2.15 (-1.77, 6.08), p=0.20 ♀ 1.81 (-3.19, 6.83), p=0.30	♂ 1.00 (-0.69, 2.69), p=0.13 ♀ 0.74 (-1.18, 2.65), p=0.33			
20-59 years (n= 250♂; 199♀)	♂ 4.60 (-0.03, 9.24), p=0.08 ♀ 3.13 (-0.73, 6.99), p=0.09	♂ 1.74 (-0.28, 3.76), p=0.10 ♀ 1.08 (-0.75, 2.92), p=0.29			
60-80 years (n= 93♂; 85♀)	♂ 0.68 (-7.42, 8.78), p=0.83 ♀ -2.82 (-8.89, 3.25), p=0.38	♂ 0.41 (-2.47, 3.28), p=0.85 ♀ -0.96 (-4.04, 2.11), p=0.53			
adult Mexican non-diabetic (n=182)	Spearman correlation ♀ 0.1843*	Spearman correlation ♀ 0.0666, NS	Spearman correlation ♀ 0.1982*		Svensson et al. (2010)
MEHOP					
>20 years	NHANES 1999-2002, adjβ(SE) n=1,292 ♂ 1.79 (0.55), p= 0.005*			NHANES 1999-2002, adjβ(SE) n=622 ♂ 0.044 (0.027), p=0.125	Stahlhut et al. (2007) ¹
	NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend	NHANES 1999-2002, adjβ(SE) Q4 vs Q1 (referent), p-trend			Hatch et al. (2008) ²
6-11 years (n= 97♂; 86♀)	♂ 0.60 (-2.68, 3.88), p=0.77 ♀ 0.45 (-5.56, 6.46), p=0.97	♂ 0.14 (-1.21, 1.48), p=0.91 ♀ -0.17 (-2.60, 2.26), p=0.79			
12-19 years (n= 161♂; 171♀)	♂ 0.68 (-2.67, 4.02), p=0.76 ♀ 1.79 (-4.10, 7.68), p=0.37	♂ 0.27 (-1.40, 1.94), p=0.65 ♀ 0.89 (-1.40, 3.18), p=0.32			
20-59 years (n= 251♂; 198♀)	♂ 5.81 (0.69, 10.94), p=0.06 ♀ 1.52 (-2.98, 6.02), p=0.38	♂ 2.14 (-0.13, 4.41), p=0.09 ♀ 0.38 (-1.90, 2.66), p=0.62			
60-80 years (n= 92♂; 83♀)	♂ 2.31 (-4.97, 9.59), p=0.60 ♀ 2.46 (-7.41, 12.32), p=0.71	♂ 0.69 (-2.05, 3.44), p=0.75 ♀ 0.94 (-2.98, 4.85), p=0.80			
adult Mexican non-diabetic (n=182)	Spearman correlation ♀ 0.0865, NS	Spearman correlation ♀ -0.0111, NS	Spearman correlation ♀ 0.1123, NS		Svensson et al. (2010)
MiBP					
adult Mexican non-diabetic (n=182)	Spearman correlation ♀ 0.0151, NS	Spearman correlation ♀ 0.0457, NS	Spearman correlation ♀ -0.0156, NS		Svensson et al. (2010)
MCPP					
adult Mexican non-diabetic (n=182)	Spearman correlation ♀ -0.0475, NS	Spearman correlation ♀ -0.0686, NS	Spearman correlation ♀ 0.0728, NS		Svensson et al. (2010)

Appendices

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Appendix Table A. Association between urinary phthalate metabolites and indicators of obesity or insulin resistance.

Urine Metabolite	Waist Circumference (cm)	Body Mass Index, BMI (kg/m ²)	Waist Height Ratio	HOMA (ln)	Reference
MECPP					
adult Mexican non-diabetic (n=182)	Spearman correlation ♀ 0.1302, NS	Spearman correlation ♀ 0.0301, NS	Spearman correlation ♀ 0.1235, NS		Svensson et al. (2010)
ΣDEHP metabolites³					
adult Mexican non-diabetic (n=182)	Spearman correlation ♀ 0.1427, NS	Spearman correlation ♀ 0.0354, NS	Spearman correlation ♀ 0.1498*		Svensson et al. (2010)

¹Stahlhut et al. (2007) adjusted for age, race/ethnicity, total fat and calorie intake, physical activity level, smoking exposure, urine creatinine, glomerular filtration rate, ALT, and GGT

²Data from Hatch et al. (2008) presented in “additional material” files. Adjusted for age, creatinine, height, race/ethnicity, socioeconomic status, % of daily calories from total fat (tertiles), daily servings of dairy (tertiles), daily servings of fruit and vegetables (tertiles), METS/month (continuous) (age 12+), TV/video/computer use (≤ 1 hour/day, >1 and <2.5 hours/day, ≥ 2.5 hours/day), smoking status (age 20+), and menopausal status and parity (women age 20+).

³DEHP metabolites = MEHP, MEHHP, MEOHP, and MECPP

*p <0.05

Appendices

(version updated January 5, 2011)

Appendix Table B. Summaries of Organotin Studies in Experimental Animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Summary of key findings	Reference
Pregnant C57BL/6 mice received TBT chloride by daily ip injection at 0, 0.05 or 0.5 mg/kg bw/day from GD12 – GD18 (sesame oil vehicle control). Neonates were killed on PND1 and sections of liver, testis, mammary and adipose tissue were prepared for histopathological evaluation; slides were stained with Oil Red O (for lipids) and hematoxylin.	10 dams per group	0.05 TBT chloride (ip injection) 0.5	Disorganization of hepatic and gonadal architecture and significantly increased Oil Red O staining in treated animals vs. controls (this effect was more severe at the higher dose level). Liver sections exhibited signs of steatosis consistent with dysregulation of fatty acid uptake. Oil Red O positive staining in mammary and inguinal adipose tissues was dramatically elevated, reflecting either an increase in lipid accumulation or an increase in mature adipocytes.	Grun et al. (2006)
Pregnant C57BL/6 mice received TBT chloride by ip injection at 0 or 0.3 mg/kg bw/day from GD12 through delivery (sesame oil vehicle control). Pups were cross fostered to untreated dams and followed for 10 weeks. Males were killed on PND70 and epididymal fat pads were weighed.	10 pups/sex per group	0.3 TBT chloride (ip injection)	↑ weight of epididymal fat pad (1.20-fold) no effect findings: Body weight for either sex up to PND70	
C57BL/6 male mice (6-weeks old) received TBT chloride as a single ip injection at 0 or 0.3 mg/kg bw (sesame oil vehicle control). Animals were killed after 24 hours; cDNA was prepared from liver, epididymal fat pad, or testis for quantitative real-time PCR analysis. <u>Also presented in study, but not summarized here:</u> Plasmids and transfections; ligand binding; 3T3-L1 cell assays. Mouse experiments with AGN195203 or troglitazone.	3 per group	0.3 TBT chloride (ip injection)	↑ Expression Liver: CEBPα, CEBPβ, Fatp, Acac Adipose: RXRα, Fatp, Pck1 Testis: CEBPβ, Pck1 ↓ Expression Liver: PPARγ Adipose: CEBPα, CEBPδ no effect findings, expression of: Liver: RXRα, CEBPδ, Srebf1, Pck1, Fasn Adipose: PPARγ, CEBPβ, Srebf1, Acac, Fasn Testis: RXRα, PPARγ, CEBPα, CEBPδ, Srebf1, Acac, Fasn	
<i>Xenopus laevis</i> tadpoles treated with 1-10nM TBT from stage 48 to metamorphs	9 per group	1-10 nM TBT (water)	↑ ectopic adipocyte formation posterior to the fat bodies in and around the gonads of both sexes (dose-dependent) *10 nM AGN195203 , and 1 μM troglitazone also produced ectopic adipocytes in ~45-60% animals no effect findings: sex ratio, time to metamorphosis, or gross structure of liver or kidney.	
Pregnant C57BL/6 mice received TBT by gavage with 0 or 0.1 mg/kg bw/day TBT or 1 mg/kg rosiglitazone (ROSI) on GD16.5 (0.5% carboxymethyl cellulose vehicle control). Offspring were analyzed on delivery (sesame oil vehicle control). Pups were killed at 8 weeks of age and stromal stem cells were isolated from white epididymal/ovarian fat pads (WAT) <u>Also presented in study, but not summarized here:</u> In vitro studies on adipose-derived stromal stem cells (ADSCs) derived from untreated 8-week old mice	~ 5 per group	0.1 TBP (gavage)	↑ lipid accumulation in isolated ADSCs incubated in DMSO (~2-fold); effect was enhanced when isolated ACSCs were incubated in 500 nM ROSI or 50 nM TBT ↑ gene expression of Fabp4 and LEP (based on target:housekeeping ratio) in isolated ADSCs treated with DMSO; effect was enhanced when isolated ACSCs were incubated in 500 nM ROSI or 50 nM TBT ↑ number of preadipocytes ↑ number of ADSCs that became adipocytes	Kirchner et al. (2010)

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Appendix Table B. Summaries of Organotin Studies in Experimental Animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Summary of key findings	Reference
Japanese white rabbits (male, 3-5 months old); single dose of 0 or 100 mg/kg bw TPT fluoride (25% of LD ₅₀) in sesame oil vehicle by oral gavage after 12-hour fasting; animals were followed for a period of 21 days.	5 per group for most endpoints (range of 3-10)	100 TPT fluoride (gavage)	<p>↓ fasting insulin and ↑ fasting glucose, triglycerides, total cholesterol and phospholipid (back to control levels by day 10 post-dosing)</p> <p>↓ plasma and adipose LPL activity, most apparent at days 3-7 post-dosing</p> <p>↑ plasma chylomicron, VLDL, intermediated density lipoprotein, LDL, and total lipoprotein</p> <p>↓ dietary intake in week after treatment but no effect on body weight</p> <p>↓ glucose-stimulated insulin release and impaired IVGTT</p> <p>↓ glucagon-stimulated insulin release and elevated glucose after IV glucagon challenge</p> <p>↓ arginine-stimulated insulin and glucagon release and elevate glucose after IV arginine challenge</p> <p>no effect findings: response to insulin tolerance test; plasma HDL; islet cell or exocrine pancreas histopathology</p>	Manabe and Wada (1981)
Multiple species comparison (in males) of hyperglycemic response to a single oral dose TPT hydroxide (administered in sesame oil suspension), assessment 3 days later: Japanese white rabbit (0 or 100 mg/kg); golden hamster (0 or 100 mg/kg); Wistar rat (0 or 200 mg/kg); ddY mouse (0 or 100 mg/kg); Hartley guinea pig (0 or 100 mg/kg)	5-8	100 TPT hydroxide (Japan white rabbit, hamster, ddY mouse, Hartley guinea pig) (oral) 200 (Wistar rats)	<p>↑ plasma glucose in rabbits (3.24-fold) and hamsters (2.93-fold) but not ddY mice or guinea pigs; ↓ body weight in hamsters, mice, guinea pigs</p> <p>↔ plasma glucose; ↓ body weight</p> <p>no effect findings in any treatment group: islet cell morphology</p>	Matsui et al. (1984)
Golden hamsters (male); single gavage dose of 0 or 6 mg/100g bw [60 mg/kg] of TPT chloride in sesame oil; animals assessed on day 1, 2, 3, 4, and 7 after treatment	6-8 per group per time point	60 TPT chloride (gavage)	<p>↑ fasting plasma glucose on days 1 (2.1-fold) and 2 (1.8-fold, but not statistically significant)</p> <p>↓ basal IRI on days 1 and 2 (46 to 58% of control)</p> <p>↓ ΔIRI/ΔPF on day 1 (30% of control)</p> <p>↑ fasting triglycerides on day 1 (7.56-fold) and 2 (3.16-fold, but not statistically significant)</p> <p>Effects were transient and there were no differences in treated animals on any of these endpoints by day 3 post-treatment. Pancreatic levels of TPT at day 3 were 0.64 μg/g tissue compared to 1.43 μg/g on day 1 and were undetectable prior to TPT treatment (day 0).</p> <p>no effect findings: pancreatic histopathology or lymphocyte infiltration induced by pancreatitis</p>	Ogino et al. (1996)
Golden hamsters (male; ≥ 4 weeks); dietary dose levels of 1.28, 28.82, or 54.77 mg TPT chloride per 1 kg feed (≈ 10.8, 220.1, and 383.2 μg TPT/day) [≈ 0.072, 1.47, and 2.22 mg/kg/day]. Hamsters were treated for 30, 60, 120, or 180 days.	2-4	0.072 TPT chloride (diet) 1.47 2.22	<p>no effects observed</p> <p>↓ body weight gain</p> <p>↓ body weight gain</p> <p>no effect findings: plasma glucose measured at 30, 60, 120, or 180 days</p>	Ohhira et al. (1996)
Male golden hamsters (6-weeks old) received a single dose of TPT chloride by oral gavage at 50 mg/kg [there was no control for this dose group; this was a pre- and post-treatment time course study]. Animals were killed immediately pre, and at 3, 6, 12, 24, 48, 72 and 96 hours post dose; blood and liver were collected.	32 (4 per time point)	50 TPT chloride (gavage)	<p>↑ plasma glucose at 12 hours (~2.4-fold over baseline), 24 hours (~2.2-fold over baseline), 48 hours (~2.3-fold over baseline), and 72 hours (~1.5-fold over baseline)</p> <p>↑ plasma triglycerides at 24 hours (~5.0-fold over baseline) and 48 hours (~6.7-fold over baseline); no effect at earlier time points of 72 and 96 hours</p>	Ohhira et al. (1999)

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Appendix Table B. Summaries of Organotin Studies in Experimental Animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Summary of key findings	Reference
<p>Plasma glucose, triglycerides and glutamic oxaloacetic transaminase (GOT), and hepatic cytochrome P-450 levels were determined.</p> <p><u>Also presented in study, but not summarized here:</u> hepatic CYP levels; plasma GOT; tissue levels of TPT, mono- and di- phenyltin, and inorganic tin, monophenyltin, diphenyltin in animals pretreated with phenobarbital; detailed findings in animals pre-treated phenobarbital, β-naphthoflavone, and 3-methyl-cholanthrene</p>			<p>The study was designed to see if inducers of cytochrome p450s would attenuate the hyperglycemic effects of TPT in hamsters. The hyperglycemic response seen in TPT treated animals at 12-72 hours was less pronounced in animals that were co-treated with phenobarbital, an inducer of CYP2B, 2C, and 3A. Pancreatic, liver, kidney, and brain levels of TPT in phenobarbital treated animals were also lower in the 2 to 3 day period after treatment compared to TPT animals that were not co-treated with phenobarbital. Inducers of CYP1A and 2A (β-naphthoflavone and 3-methylcholanthrene) were less effective with β-naphthoflavone showing no attenuation of TPT-induced hyperglycemic response.</p>	
<p>ddY mice (male, young adult, \geq 8 weeks); single oral dose of 0 or 50 mg/kg TPT chloride in sesame oil; some animals pre-treated with a single dose of 50 mg/kg SKF-525A, an inhibitor of cytochrome P450s</p>	8	50 TPT chloride (oral)	<p>\uparrow plasma glucose in mice pre-treated with SKF-525A, but not in animals without the pre-treatment.</p> <p>Liver and pancreatic levels of TPT were higher in mice treated with SKF-525A; suggesting that the reported relative insensitivity of mice to become hyperglycemic after TPT treatment is related to tissue accumulation</p> <p>no effect findings: plasma triglyceride; liver toxicity</p>	Ohhira et al. (2000)
<p>Male Japanese white rabbits received a single dose of triphenyltin (TPT) chloride by oral gavage (sesame oil vehicle control) at 0 or 100 mg/kg bw.</p> <p><u>Also presented in study, but not summarized here:</u> Results with alloxan-diabetic rabbits; tissue TPT levels; detailed findings in animals treated with tolbutamide and TPT</p>	6/group	100 TPT chloride (gavage)	<p>\uparrow plasma fasting glucose (2.39-fold) \uparrow plasma triglycerides (6.07-fold) \downarrow ratio of immunoreactive insulin (IRI):fasting plasma glucose</p> <p>The experiment was designed to determine whether the reported effects of TPT on hyperglycemia could be mediated through interactions with K-ATP channels by comparing the response to the sulfonylurea tolbutamide in control and TBT-treated mice. The effects on insulin secretion from tolbutamide were similar in both groups which led the authors to conclude that TBT was exerting its hyperglycemic effects before the process of membrane depolarization due to interactions of K_{ATP} and sulfonylurea receptors.</p> <p>no effect findings: basal IRI</p>	Watanabe et al. (2002)
<p>Male KM mice (21 days old) treated with TBT chloride by oral gavage once every three days at 0, 0.0005, 0.005, or 0.05 mg/kg bw (vehicle control is 0.15% ETOH in saline). After 45 days of treatment the mice were fasted overnight, liver weights and hepatic, renal and testicular fat pad weights were recorded. Liver sections were stained with Oil red O [for lipids] and examined histopathologically. Plasma glucose, leptin and insulin were measured; plasma and hepatic adiponectin and resistin concentrations were determined.</p>	6-8/group	<p>0.0005 TBT chloride (every third day) (gavage)</p> <hr/> <p>0.005 (every third day)</p> <hr/> <p>0.05 (every third day)</p>	<p>\uparrow plasma leptin (~1.3-fold) \downarrow plasma adiponectin (~64% of control) \downarrow hepatic adiponectin (~87% of control) \downarrow hepatic resistin (~74% of control)</p> <hr/> <p>\uparrow body weight gain (1.09-fold) \uparrow fat weight/body weight (1.37-fold) \uparrow plasma insulin (1.84-fold) \uparrow plasma leptin (1.57-fold) \uparrow plasma resistin (1.59-fold) \downarrow hepatic adiponectin (~82% of control) \downarrow hepatic resistin (~68% of control)</p> <hr/> <p>\uparrow plasma leptin (~1.3-fold) \downarrow hepatic adiponectin (~72% of control)</p>	Zuo et al. (2009)

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Appendix Table B. Summaries of Organotin Studies in Experimental Animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/day)	Summary of key findings	Reference
			Administration of TBT resulted in severe hepatocyte cytoplasmic degeneration - the cytoplasm appeared as ghost structures and congestive sites were observed. Examination revealed the presence of lipid droplets whose bulk dramatically increased as the TBT doses increased.	
			no effect findings: feed consumption; plasma glucose; hepatosomatic index	
Abbreviations: IRI = immunoreactive insulin; TBT = tributyltin; TPT = triphenyltin; SKF-525A = α -phenyl- α -propylbenzeneacetic acid 2-[diethylamino]-ethyl ester hydrochloride				

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Appendix Table C. Summaries of Phthalate Studies in Experimental Animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects	Reference
<p>Pregnant Wistar rats treated with 0 or 600 mg/kg bw/d DiBP by oral gavage from GD7-21; offspring assessed at GD19 or GD21</p> <p>For datasets including more than one animal per litter, the litter was the statistical unit and included in the mixed model analysis of variance as an independent, random factor</p> <p>*insulin and leptin based on pooled samples from 1 or 2 litters per sex ($n = 6-8$). Data from 3-4 males and 3-4 females per litter were analyzed together as no sex difference in levels of these hormones was found.</p>	8 dams per group	600 DiBP (gavage)	<p>↓ plasma insulin (~70% of control)*</p> <p>↓ plasma leptin (~50% of control)*</p> <p>↓ fetal body weight on GD19 (a 10% reduction on GD21 was not statistically significant)</p> <p>↓ PPARα mRNA in livers and testes of male fetuses on GD19 but not GD21</p> <hr/> <p>no effect findings: plasma levels of MCP1, IL-1B, PAI-1 active, IL6, or TNF; ovarian levels of PPARα and PPARγ mRNA; PPARγ mRNA in liver (control levels low)</p>	Boberg et al. (2008)
<p>Male and female C57B16/J mice. Males received DEHP in the diet at 0 or ~200 mg/kg bw/d/day for 3 weeks prior to mating with untreated females (sunflower used as vehicle); females were fed the same diets for three weeks prior to mating with untreated males, through gestation and lactation. At weaning, F1 offspring were fed a normal control diet until they were 7 weeks old when they were switched to a high fat diet for 12 weeks. Dam number and control for litter effects not reported.</p>	8 F1 males	~200 DEHP (diet) 2 separate parental treatment groups: F0 males or females	<p>no effect findings for F1 generation: body weight, liver weight, brown and white adipose tissue weights and blood glucose levels at 3 weeks in F1 offspring; body weight gain from 7 to 19 weeks on standard or high fat diet</p>	Casals-Casas et al. (2008)
<p>Male WT (wild type) C57B16J mice fed DEHP in a normal diet at 0, 100 or 1000 mg/kg bw/day for 13 weeks. Fat and lean body composition, and plasma lipids and glucose were measured.</p>	10 per group	100 DEHP (diet) 1000 DEHP	<p>↓ Plasma triglycerides, week 13 (~88% of control)</p> <hr/> <p>↓ Body weight change, week 13 (~85% of control)</p> <p>↓ Fat body mass, week 13 (~57% of control)</p> <p>↓ Epididymal fat pad weight, week 13 (~60% of control)</p> <p>↑ Relative liver weight, week 13 (~1.42-fold)</p> <p>↓ Plasma triglycerides, week 13 (~41% of control)</p> <p>↓ Plasma free fatty acids, week 13 (~67% of control)</p> <p>↑ Total plasma ketone bodies, week 13 (~1.7-fold)</p> <p>↓ Plasma insulin, week 13 (~57% of control)</p> <hr/> <p>No effect findings at any dose level: Feed consumption; lean body mass; plasma glucose</p> <hr/> <p>↓ Body weight change, week 5 (~90% of control) through week 13</p>	Feige et al. (2010)
<p>Male WT (wild type) C57B16J mice fed DEHP in a</p>	9	500 DEHP (diet)	<p>↓ Body weight change, week 5 (~90% of control) through week 13</p>	

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Appendix Table C. Summaries of Phthalate Studies in Experimental Animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects	Reference
<p>high fat diet at 0 or 500 mg/kg bw/day for 13 weeks. Fat and lean body composition and plasma lipids were measured. Metabolic phenotyping and an IPGTT were conducted. Gene expression was measured in well-characterized PPAR target genes.</p> <p><u>Also presented in study, but not summarized here:</u> PPARα and PPARβ activity; effects of DEHP on PPARα WT and PPARα-humanized mice (effects discussed in text)</p>			<p>(~85%)</p> <p>↓ Fat body mass, week 13 (~57% of control)</p> <p>↓ Epididymal fat pad weight, week 13 (~58% of control)</p> <p>↑ Relative liver weight, week 13 (~1.36-fold)</p> <p>↓ Plasma triglycerides, week 13 (~50% of control)</p> <p>↑ Total plasma ketone bodies, week 13 (~1.3-fold)</p> <p>↓ Plasma insulin, week 13 (~50% of control)</p> <p>Metabolic phenotyping:</p> <p> ↑ O₂ consumption: daytime (~1.42-fold), nighttime (~1.31-fold)</p> <p> ↑ CO₂ release: daytime (~1.28-fold), nighttime (~1.50-fold)</p> <p>enhanced glucose tolerance:</p> <p> ↓ Plasma glucose at 15 and 30 minutes during IPGTT</p> <p> ↓ AUC (97.3% of control)</p> <p>↑ Gene expression:</p> <p> Liver – MCAD, ACOX, FABP-1, CPT-1a, FGF-21</p> <p>↑ Hepatic HAD activity (1.42-fold)</p> <hr/> <p>No effect findings at any dose level: Feed consumption; lean body mass; plasma ASAT, ALAT, free fatty acids, and glucose; fecal lipid and cholesterol levels; respiratory exchange ratio, horizontal and vertical locomotor activity, distance to fatigue. Gene expression in muscle, epididymal white adipose tissue, and brown adipose tissue.</p>	
<p>Female Wistar rats; 0 or 750 μg/100 g bw [7.5 mg/kg bw/d] DEHP by ip injection on alternate days for 14 days, evaluated after 14 days</p> <p><u>Also presented in study, but not summarized here:</u> Human blood was stored in glass bottles, glass bottles with DEHP added, or DEHP- plasticized bags; aliquots assayed after 7, 14 and 21 days of storage.</p>	8	7.5 DEHP (ip injection)	<p>↑ blood glucose (1.12-fold); ↓ liver glycogen (87% of control); ↓ serum insulin (87% of control); ↑ serum T3 (1.6-fold) and T4 (1.2-fold); ↓ serum cortisol (90% of control).</p> <hr/> <p>no effect findings: TSH; no effect on any endpoint listed above in animals in a 7-day recovery group</p>	Gayathri et al. (2004)

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Appendix Table C. Summaries of Phthalate Studies in Experimental Animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects	Reference
Male and female common marmosets received DEHP by oral gavage (catheter) at 0 (corn oil vehicle), 100, 500, or 2500 mg/kg bw/d for 13 weeks. Clinical chemistry parameters were assessed at 4 and 13 weeks. At the end of the treatment period, the animals were killed; routine organs were weighed and a full tissue list was collected for histopathological evaluation.	4/sex per group	100, 500, or 2,500 DEHP (gavage)	no effect findings: pancreatic weight or light microscopic findings	Kurata et al. (1998)
<u>Also presented in study, but not summarized here:</u> Data from clofibrate (positive control) group, results of preliminary study; testicular findings; blood cholecystokinin, testosterone, and estradiol analyses; peroxisome morphometry; hepatic peroxisomal enzyme activity and p-450 content; testicular zinc analysis				
Wistar male rats fed DEHP in the diet at 0 or 2 % for 21 days (daily dose levels were estimated to be 0 or ~1600 mg/kg bw/d). At the end of the treatment period, the rats were killed and isolated adipocytes were prepared from the lumbar and epididymal fat. Fat cell size was determined, and lipoprotein lipase (LPL) and lipolytic activity were measured.	6 per group	~1600 DEHP (diet)	<p>↓ body weight (83% of control) and body weight gain (66% of control)</p> <p><u>Epididymal adipose tissue:</u> ↓ weight (74% of control) ↑ LPL activity (~1.52-fold)</p> <p><u>Lumbar adipose tissue:</u> ↓ weight (67% of control) ↑ LPL activity (~1.61-fold)</p> <p><u>Isolated Epididymal Adipocytes:</u> ↓ cell diameter (93% of control) and cell volume (78% of control) ↓ triglyceride content (89% of control)</p> <p>no effect findings: feed intake; distribution of adipocyte cell means; basal and stimulated lipolysis in isolated adipocytes.</p>	Martinelli et al. (2010)
Adult male Wistar rats; 0 or 2% DEHP in the diet for 21 days [estimated to be ~1000 mg/kg bw/day]	not reported	1000 DEHP (diet)	<p>OGTT: ↑ AUC (1.22-fold), higher glucose levels throughout the test with no normalization after 180 minutes; ↓ liver G-6-P (42% of control), ↓ G-1-P (63% of control), ↓ F-6-P (61% of control), and ↓ glycogen (81% of control); ↓ muscle G-6-P (48% of control).</p> <p>no effect findings: liver and muscle F-1, 6-P₂ or glucose levels; muscle F-6-P, G-1-P, or glycogen levels.</p>	Martinelli et al. (2006)
<u>Also presented in study, but not summarized here:</u> Muscle, liver and circulating metabolite levels: TG, ATP, creatinine, phosphate creatinine, lactate, pyruvate, and/or citrate.				

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Appendix Table C. Summaries of Phthalate Studies in Experimental Animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects	Reference
Male Crlj:CD1(ICR) mice (6 weeks old) fed DEHP in the diet at 0 or 2% for 10 days. [Daily dose is estimated to be ~2400 mg/kg bw/d]. On day 10, blood was collected and testes, liver, kidney and pancreas were weighed.	7	~2400 DEHP (diet)	↓body weight (76% of control) ↓pancreas weight (~91% of control)	Miura et al. (2007)
<u>Also presented in study, but not summarized here:</u> Lipid peroxidation; serum biochemistry, mineral components of testes; tissue MEHP and DEHP concentrations; liver weight, water consumption tissue NO concentrations.				
adult Male albino rats received saline of DEHP by ip injection on days 1, 5 and 10 at 0 or 5 mL/kg bw/day [dose level as mg/kg bw/d was not reported]; an OGTT was conducted (n=9/group); the rate of glycogenesis was estimated 3 hours after a glucose challenge (n=12/group); and rate of glycogenolysis was estimated 3, 5, 7, 9 and 11 hours after a glucose challenge (n=6/group). Rats were killed on day 22, blood and liver were collected. Liver glycogen was estimated (n=6/group); and carbohydrate metabolism enzyme activities were measured (n=6/group).	6-12/endpoint	5 mL/kg-d DEHP [DEHP dose per kg bw cannot be calculated in this paper, only the volume of the injection was presented]	impaired glucose tolerance in OGTT (2 g glucose/kg body weight) ↓ liver glycogen content in non-fasted rats (27% of control) ↑ liver glycogen content in fasted rats (1.30-fold) ↓ glycogenesis 3 hours after glucose (41% of control) ↓ glycogenolysis 3 hours (43% of control), 5 hours (32%), 7 hours (56%), and 9 hours (78%) after glucose carbohydrate metabolism enzyme activity: ↓G6-Pase (53% of control) ↓phosphorylase (76% of control) ↓glucose-6-phosphate (64% of control) no effect findings: feed consumption; FdPase and Aldolase activity	Mushtaq et al. (1980)
<u>Also presented in study, but not summarized here:</u> U- ¹⁴ C-labelled glucose assays into hepatic glycogen				
adult Male Wistar rats; 0, 1, 2, 4% DEHP in diet [daily dose levels not stated, estimated at 0, 500, 1000, and 2000 mg/kg bw/day] , terminated after 1, 2, or 4 weeks exposure (n=10 rats/time point for controls, n=5 rats/ time point for DEHP-treated groups).	5 per group	500 DEHP (diet)	↓ Plasma triglycerides (49 – 55% of control during weeks 1 and 2, 77-80% of control during week 4); ↑ free plasma fatty acid (1.4 – 2.9-fold) and blood ketone bodies (1.4 – 5.3-fold)	Sakurai et al. (1978)
		1000 DEHP	Decreased body weight gain (high-dose lost weight) and food consumption (~33% of control in high-dose); marked decrease in epididymal fat pad weights [data not shown] ; blood glucose (~80% of control); Increased liver weight (1.6 – 2.0-fold at 2 weeks); ↓ liver glycogen (38% of control)	
		2000 DEHP	Decreased body weight gain (high-dose lost weight) and food consumption (~33% of control in high-dose); marked decrease in epididymal fat pad weights [data not shown] ; blood glucose (~80% of control); Increased liver weight (1.6 – 2.0-fold at 2 weeks); ↓ liver	

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Appendix Table C. Summaries of Phthalate Studies in Experimental Animals

Species, strain, and experimental design	Sample size	Dose (mg/kg bw/d)	Effects	Reference
			glycogen (38% of control)	
			Not affected at and dose level: plasma cholesterol	
Female Sprague-Dawley rats were obtained from parents that had been maintained on soy-free diets; the experimental females were also maintained on soy-free diets. At 3 months of age, the females were ovariectomized and then placed on diets containing dibutylphthalate (DBP) at 0, 83, or 393 mg/kg bw/d for 12 weeks. At the end of the treatment period the animals were killed, the size of the paratibial fat deposit was measured and blood was collected.	12 per group	82 DEHP (diet)	↑paratibial fat mass (~1.15-fold) ↓feed consumption (93%)	Seidlova-Wuttke et al. (2005)
		393 DEHP	↑paratibial fat mass (~1.14-fold) ↓feed consumption (89%) ↓serum LDL (~73% of control) ↓serum T3 (~85% of control)	
<u>Also presented in study, but not summarized here:</u> Effects of BPA, BP2, procymidone, linurone, or E2; binding assays			no effect findings: body weight gain and water consumption; serum leptin, triglycerides, HDL, cholesterol; serum TSH, T ₄ , luteinizing hormone	

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Appendix Table D. Comparison of phthalate monoester activities for PPAR α , PPAR β , and PPAR γ and effects on adipogenesis (Bility 2004)

Appendix Table D. Comparison of phthalate monoester activities for PPAR α , PPAR β , PPAR γ in transfected 3T3-L1 cells and ability to induce adipogenesis in 3T3-L1 fibroblasts														
monoester	structure	mouse PPAR α		human PPAR α		mouse PPAR β		human PPAR β		mouse PPAR γ		human PPAR γ		adipogenesis at 50 μ M*
		LOAC (μ M)	max fold-induction	LOAC (μ M)	max fold-induction	LOAC (μ M)	max fold-induction	LOAC (μ M)	max fold-induction	LOAC (μ M)	max fold-induction	LOAC (μ M)	max fold-induction	
MEP		--	--	--	--	--	--	--	--	--	--	10**	3.3	not tested
MButP		100	3.7	200	2.4	3	4.7	--	--	--	--	--	--	"no induction"
MBenP		100	12.3	200	2.5	100	10.8	--	--	100	7.8	200	4.2	"no induction"
MEHA		100	8.3	100	2.7	100	4.2	--	--	30	5.3	--	--	not tested
MIHP2		30	8.2	200	5.6	30	8.3	--	--	200	3.6	--	--	not tested
MEHP		10	11.1	30	4.8	200	16.8	--	--	30	2.5	10	5.5	"strong" induction
MIHP		10	26.1	30	12.3	200	2.2	--	--	100	5.4	100	6.7	not tested
MnOP		10	32.4	10	7.6	100	13.7	--	--	10	11.0	100	19.1	"strong" induction
MINP		3	27.1	10	5.8	--	2.8	--	--	3	14.1	30	9.3	"strong" induction
MIDP		3	26.9	30	3.9	100	7.6	--	--	30	4.2	3	8.1	"strong" induction

--- not active; LOAC = lowest activation concentration

From Bility et al. (2004); Lipid accumulation was measured using Oil Red O staining 3T3-L1 fibroblasts were cultured with DMEM/4% FBS containing 1.0 μ g/ml insulin, 1 μ M dexamethasone and 100 μ M isomethylbutylxanthine under the following treatment conditions (A) DMSO (vehicle); (B) Troglitazone, 10 μ M; (C) MBut, 50 μ M; (E) MBenP, 50 μ M; (F) MEHP, 50 μ M; (G) MINP, 50 μ M; (H) MIDP, 50 μ M; and (I) MnOP, 50 μ M. The media was replaced with DMEM/4% FBS containing 1.0 mg/ml insulin and troglitazone (10 mM), or the indicated phthalate monoester every 48 h after initiation of differentiation. The cells were fixed 6 days after initiation of adipogenesis and stained with Oil Red O.

MEP = monoethyl phthalate; MButP = monobutyl phthalate; MBenP = monobenzyl phthalate; MEHA = mono-2-ethylhexyl adipate; MIHP = monoisoheptyl phthalate; MEHP = mono-2-ethylhexyl phthalate; MnOP = mono-n-octyl phthalate; MINP = monoisononyl phthalate; MIDP = monoisodecyl phthalate

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Appendix Table E. Comparison of phthalatmonoester activities for PPAR α , PPAR γ , and effects on adipogenesis (Hurst 2003)

Appendix Table E. Comparison of phthalate monoester activities for PPAR α and PPAR γ in transfected COS cells and ability to induce adipogenesis in 3T3-L1 fibroblasts

monoester	mouse PPAR α		human PPAR α		mouse PPAR γ		human PPAR γ		adipogenesis
	LOAC, μ M (EC ₅₀)	max fold-induction	LOAC, μ M (EC ₅₀)	max fold-induction	LOAC, μ M (EC ₅₀)	max fold-induction	LOAC, μ M (EC ₅₀)	max fold-induction	
MMP	not reported	~2	--	--	not reported	not reported	not reported	not reported	
M(n)BuP	--	--	--	--	not reported	not reported	not reported	not reported	
DMP	--	--	--	--	not reported	not reported	not reported	not reported	
DEP	--	--	--	--	not reported	not reported	not reported	not reported	
DEHP									
M-sec-ButP	10 (63 μ M)	~3	not reported	not reported	300	~2	NR	NR	dose-dependent increase (100 and 300 μ M)
MEHP	0.5 (0.6 μ M)	~2.5	4 (3.2 μ M)	~2.3	3 (10.1 μ M)	~3.7	5 (6.2 μ M)	~2.8	50 μ M, strong
MBzP	10 (21 μ M)	~4.6	30 (30 μ M)	~2.3	10 (75 μ M)	~4	30 (100 μ M)	~4	dose-dependent increase (100 and 300 μ M)

--- not active; NR = not reported; LOAC = lowest activation concentration; maximal fold induction is compared to DMSO treated controls

From Hurst et al. (2003)

Activation of PPAR α and PPAR γ in COS-1 cells transfected with Activation of mouse and human PPAR α and PPAR γ based on luciferase reporter values normalized to untreated DMSO controls. COS-1 cells were transfected with expression plasmid encoding the indicated PPAR isoform, a PPRE-Luc reporter plasmid, and a renilla luciferase internal control plasmid. Cells were treated for 24 hours and firefly luciferase activity was normalized to the renilla luciferase internal control.

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Appendix Table F. Comparison activities of several environmental chemicals for PPAR α and PPAR γ in transfected COS cells

monoester	mouse PPAR α		human PPAR α		mouse PPAR γ		human PPAR γ	
	LOAC (μ M)	max fold-induction	LOAC (μ M)	max fold-induction	LOAC (μ M)	max fold-induction	LOAC, μ M	max fold-induction
PFOA	0.5	17.2	1	8.6	--	--	20*	1.3
Phenoxyacetic acid herbicides								
2,4-D	--	--	--	--	--	--	--	--
MCPA	50*	2.1	--	--	--	--	--	--
TCE, PCE, and their metabolites								
TCE	--	--	--	--	not reported	not reported	not reported	not reported
TCE-OH	--	--	--	--	not reported	not reported	not reported	not reported
PCE	--	--	not reported	not reported	not reported	not reported	not reported	not reported
CH	not reported	not reported	not reported	not reported	--	--	not reported	not reported
TCA	1,000	3.7	1,000	3.7	100	4.5	--	--
DCA	1,000	3.5	1,000	3.7	--	--	--	--
DEHP and its metabolites								
DEHP	--	--	--	--	--	--	--	--
MEHP	5	4.2	5	3.1	5	3.7	1	3.2
EHA	10	6.2	100	3.4	--	--	--	--
EHO	--	--	--	--	--	--	--	--

--- not active; NR = not reported; LOAC = lowest activation concentration; maximal fold induction is compared to DMSO treated controls

From Maloney et al. (1999)

Wy-14,643 was used as a positive control for PPAR α activation and troglitazone for PPAR γ activation

2,4-D = 2,4-dichlorophenoxyacetic acid; CH = chloral hydrate; DCA = dichloroacetic acid; DEHP = di(2-ethylhexyl)phthalate; EHA = 2-ethylhexanoic acid; EOH = 2-ethylhexanol; MCPA = 2-methyl-4-chlorophenoxyacetic acid; MEHP = mono-2-ethylhexylphthalate; PCE = tetrachloroethylene; PFOA = perfluorooctanoic acid; TCA = trichloroacetic acid; TCE = trichloroethylene; TCE-OH = trichloroethanol

Tested concentrations: TCE (1,000 and 5,000 μ M); TCE-OH (100 and 1,000 μ M); PCE (5,000 μ M); CH (40, 100, and 1,000 μ M); TCA and DCA (100, 1,000 and 5,000 μ M); DEHP (500, 1,000 and 2,000 μ M); MEHP (0.1, 0.5, 1, 5 and 20 μ M for PPAR α plus 50 and 100 μ M for PPAR γ); EHA (10, 50, 100, 250, and 500 μ M); EHO (100, 250, 500 μ M); PFOA (0.5, 1, 5, 10, 20, 40 μ M); 2,4-D (200, 400, 800 μ M); MCPA (50, 100, 200, 400 μ M)

*significant activity not necessarily observed at higher concentrations

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Appendix Table G. ToxCast™ results for the organotin fentin (CASRN 76-87-9)					
ToxCast™ Assay	Gene Symbol	Official Full Name	AC₅₀ (μM)	E_{max}	UOM
ATG PPRE CIS	PPARA	peroxisome proliferator-activated receptor alpha	0.01	5.4	FC
ATG PPRE CIS	PPARD	peroxisome proliferator-activated receptor delta	0.01	5.4	FC
ATG PPRE CIS	PPARG	peroxisome proliferator-activated receptor gamma	0.01	5.4	FC
ATG RXRb TRANS	RXRB	retinoid X receptor, beta	0.01	2.4	FC
ATG NRF2 ARE CIS	NFE2L2	nuclear factor (erythroid-derived 2)-like 2	0.02	2.8	FC
ATG NURR1 TRANS	NR4A2	nuclear receptor subfamily 4, group A, member 2	0.02	5.3	FC
ATG PPARg TRANS	PPARG	peroxisome proliferator-activated receptor gamma	0.02	6.6	FC
NVS GPCR hDRD1	DRD1	dopamine receptor D1	0.15	95	% PC
NVS GPCR hOpiate mu	OPRM1	opioid receptor, mu 1	0.16	100	% PC
NVS GPCR hDRD2s	DRD2	dopamine receptor D2	0.17	99	% PC
NVS GPCR mCCKAPeripheral	Cckar	cholecystokinin A receptor	0.18	96	% PC
NVS GPCR h5HT7	HTR7	5-hydroxytryptamine (serotonin) receptor 7	0.20	92	% PC
NVS ENZ hPPVHR	DUSP3	dual specificity phosphatase 3	0.24	79	% PC
NVS NR hPXR	NR1I2	pregnane X Receptor	0.30	89	% PC
NVS GPCR hAdra2C	ADRA2C	adrenergic, alpha-2C-, receptor	0.43	94	% PC
NVS NR hPPARg	PPARG	peroxisome proliferator-activated receptor gamma	0.54	81	% PC
NVS GPCR hOpiate D2	DRD2	dopamine receptor D2	0.70	81	% PC
NVS GPCR hDRD4.4	DRD4	dopamine receptor D4	0.73	86	% PC
NVS GPCR hAdrb1	ADRB1	adrenergic, beta-1-, receptor	1.04	96	% PC
NVS TR hNET	SLC6A2	solute carrier family 6 (neurotransmitter transporter, noradrenalin)	1.14	85	% PC
NVS GPCR h5HT5A	HTR5A	5-hydroxytryptamine (serotonin) receptor 5A	1.84	100	% PC
NVS GPCR rNK3	Tacr3	tachykinin receptor 3	4.51	65	% PC
NVS ADME hCYP2C19	CYP2C19	cytochrome P450, family 2, subfamily C, polypeptide 19	4.64	59	% PC
NVS GPCR hAdra2A	ADRA2A	adrenergic, alpha-2A-, receptor	5.79	97	% PC
NVS GPCR hM5	CHRM5	cholinergic receptor, muscarinic 5	6.10	89	% PC
NVS ADME hCYP2C8	CYP2C8	cytochrome P450, family 2, subfamily C, polypeptide 8	6.36	62	% PC
NVS GPCR h5HT6	HTR6	5-hydroxytryptamine (serotonin) receptor 6	7.00	100	% PC
NVS ADME hCYP4F12	CYP4F12	similar to cytochrome P450, family 4, subfamily F, polypeptide 12; cytochrome P450, family 4, subfamily F, polypeptide 12	7.99	53	% PC
NVS GPCR hM1	CHRM1	cholinergic receptor, muscarinic 1	12.40	79	% PC
NVS GPCR gH2	Hrh2	histamine receptor H2	14.90	55	% PC
NVS GPCR hORL1	OPRL1	opiate receptor-like 1	20.70	64	% PC
NCGC p53	TP53	tumor protein p53	22.39	94	% PC
NVS ENZ hBACE	BACE1	beta-site APP-cleaving enzyme 1	25.70	92	% PC
NVS GPCR rmMGlur1	Grm1	glutamate receptor, metabotropic 1	26.30	76	% PC
NVS GPCR g5HT4	HTR4	5-hydroxytryptamine (serotonin) receptor 4	28.60	67	% PC
NVS GPCR rOpiateNonSelective	Oprl1	opiate receptor-like 1	42.70	65	% PC
NVS GPCR bAT2	AGTR2	angiotensin II receptor, type 2	46.70	61	% PC
NVS GPCR hM3	CHRM3	cholinergic receptor, muscarinic 3	49.70	50	% PC

UOM = unit of measure; FC = fold change; % PC = % of positive control

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