Role of Environmental Chemicals in Diabetes and Obesity: A National Toxicology Program Workshop Report

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Contributors
Michael A. Gallo served as workshop Chair and Kris Thayer was lead in organizing the meeting and assembling background materials. Jerry Heindel and John Bucher were NIEHS/DNTP staff extensively involved in organizing the meeting. Members of specific breakout groups are described in the background materials for the meeting at [http://DNTP.niehs.nih.gov/go/36433](http://DNTP.niehs.nih.gov/go/36433), see “List of Breakout Group Members.”

**Conflict of interest**

The authors declare they have no actual or potential competing financial interests.

**Abbreviations**

- As – arsenic
- BMI – body mass index
- BPA – bisphenol A
- DDE – dichlorodiphenyldichloroethylene \([1,1\text{-bis}(4\text{-chlorophenyl})-2,2\text{-dichloroethene}]\)
- DDT – dichlorodiphenyltrichloroethane \([1,1\text{-bis}(4\text{-chlorophenyl})-2,2,2\text{-trichloroethane}]\)
- DEHP – di(2-ethylhexyl) phthalate
- DERT – Division of Extramural Research and Training
- DIR – Division of Intramural Research
- DNTP – Division of the National Toxicology Program
- EPA – U.S. Environmental Protection Agency
- ERR\(\gamma\) – estrogen-related receptor \(\gamma\)
- FDA – U.S. Food and Drug Administration
- GABA – gamma-aminobutyric acid
- HOMA – homeostatic model assessment
HOMA-IR – homeostatic model assessment-insulin resistance

HTS – high throughput screening

med – median

MEP – monoethyl phthalate

MEHP – mono(2-ethylhexyl) phthalate

NCTR – FDA National Center for Toxicological Research

NHGRI – National Human Genome Research Institute

NIDDK – National Institute of Diabetes and Digestive and Kidney Diseases

NIEHS – National Institute of Environmental Health Sciences

PCBs – polychlorinated biphenyls

PNU – N-3-pyridylmethyl N'-p-nitrophenyl urea

POPs – persistent organic pollutants

ppb – parts per billion

ppm – parts per million

PPARs – peroxisome proliferator-activated receptors

PVC – polyvinyl chloride

OR – odds ratio

RXR – retinoid X receptor

T1D – type 1 diabetes

T2D – type 2 diabetes

TBT – tributyltin

TCDD – dioxin

ToxCast™ – US EPA ToxCast™ Database (http://actor.epa.gov/actor/faces/ToxCastDB/Home.jsp)
ToxRefDB – US EPA Toxicity Reference Database (http://www.epa.gov/ncct/toxrefdb/)
ABSTRACT

Background: There has been increasing interest in the concept that exposures to environmental chemicals may be contributing factors to the epidemics of diabetes and obesity. On January 11-13, 2011 the National Toxicology Program (DNTP) organized a workshop to evaluate the current state of the science on these topics of increasing public health concern.

Objective: The main objective of the workshop was to develop recommendations for a research agenda following a critical analysis of literature reports for humans and experimental animals exposed to certain environmental chemicals. The chemicals, or chemical classes, considered at the workshop were arsenic, persistent organic pollutants (POPS), maternal smoking/nicotine, organotins, phthalates, bisphenol A, and pesticides. High throughput screening data from Tox21 was also considered as a way to evaluate potential cellular pathways and generate hypotheses for testing how certain chemicals might perturb biological processes related to diabetes and obesity.

Conclusions: Overall, the review of the existing literature identified several linkages of exposures to certain chemicals or chemical classes with type 2 diabetes. There was also support for the “developmental obesogen” hypothesis which suggests that chemicals may act to alter the differentiation of adipocytes or development of neural circuits that regulate feeding behavior to result in a predisposition to obesity and related metabolic disorders, especially under the influence of high-calorie, high fat diets. Very little research was found directed towards understanding associations of environmental chemical exposures with type 1 diabetes. This lack of research was considered a critical data gap. This workshop report outlines the major themes emerging from the workshop and discusses activities that NIEHS and DNTP are undertaking to
address research recommendations. This report also serves as the introduction to a series of papers that describe in more detail the critical assessment of the literature provided by the workshop participants.
INTRODUCTION

Diabetes and obesity are major epidemics in the US and abroad. Based on data from 2005-2008, 25.6 million, or 11.3%, of all people in the US aged ≥20 years have diagnosed or undiagnosed diabetes (CDC 2011). The total direct medical costs and indirect costs (disability, work loss, premature death) associated with diabetes in the US during 2007 was $174 billion (CDC 2011). Another 35% of people in this age category have pre-diabetes, a condition where blood glucose is higher than normal but not high enough to be classified as diabetes. Diabetes is being diagnosed in individuals earlier in life as well. Being overweight or obese are well-known risk factors for the development of type 2 diabetes, perhaps accounting for ~70% of cases (Eyre et al. 2004). The prevalence of obesity worldwide had doubled since 1980 (WHO 2011). In the US, the prevalence of obesity among children and adolescents aged 2-19 years has almost tripled since 1980 and an estimated 16.9%, or 12.5 million, of children and adolescents are considered obese (Ogden and Carroll 2010). This trend is also apparent in preschool children aged 2-5 years, where obesity increased from 5% in 1976-1980 to 10.4% in 2007-2008 (Ogden and Carroll 2010).

Excess caloric consumption and a sedentary lifestyle are well-recognized risk factors for obesity and diabetes. However, there is growing consideration of “non-traditional” risk factors (e.g., environmental chemicals, stress, micronutrients, gut microbiome) as contributors in the etiology of these health conditions. Research addressing the role of environmental chemicals in diabetes and obesity has rapidly expanded in the past several years. The May 2010 White House Task
Force on Childhood Obesity (2010), the March 2011 NIH Strategic Plan for Obesity (2011), and
the February 2011 Diabetes Strategic Plan from the National Institute of Diabetes and Digestive
and Kidney Diseases (NIDDK 2011) all acknowledge the growing science base in this area and
cite the need to understand more about the role of environmental exposures as part of future
research and prevention strategies. To help develop such a research strategy the National
Toxicology Program (DNTP) organized a state-of-the-science workshop in January 2011 entitled
“Role of Environmental Chemicals in the Development of Diabetes and Obesity” to evaluate the
literature in terms of its evidence concerning associations between certain chemicals and risk of
diabetes and/or obesity. The specific chemicals or chemical classes evaluated were arsenic,
maternal smoking during pregnancy/nicotine, organic tin compounds (“organotins”), phthalates,
bisphenol A, pesticides, and various persistent organic pollutants. These are all chemicals or
chemical classes that had been linked to these diabetes and/or obesity in the literature. A diverse
group of more than 50 scientists including endocrinologists, toxicologists, epidemiologists,
bioinformaticists as well as experts in the pathobiology of diabetes and obesity were asked to
consider the current literature for consistency and biological plausibility, with the ultimate goal
of providing advice to the NIEHS/DNTP in developing a research agenda on these emerging
topics. Literature review documents, meeting presentations, and other background materials for
the workshop are available at http://DNTP.niehs.nih.gov/go/36433.

Overall, the existing literature was judged to provide plausibility, varying from suggestive to
strong, that exposure to environmental chemicals may contribute to the epidemic of diabetes
and/or obesity. This workshop report provides an overview of the major themes emerging from
the workshop and describes several activities that NIEHS and DNTP are undertaking to address
research recommendations. This report also serves as the announcement of an upcoming series of papers to be published in *Environmental Health Perspectives* describing in more detail the critical assessment of the literature provided by the workshop participants.

**WORKSHOP FORMAT AND LITERATURE SEARCH STRATEGY**

The workshop format was an introductory plenary session and a series of breakout group meetings, followed by plenary sessions to disseminate and discuss the findings from individual breakout group deliberations. A series of white papers was distributed prior to the workshop to help focus discussion. For the individual chemicals or chemical classes, workshop participants were asked to (1) evaluate the strength/weaknesses, consistency, and biological plausibility of findings reported in humans and experimental animals, (2) identify the most useful and relevant endpoints in experimental animals, *in vitro* models, and screening systems to assess these diseases, and (3) identify data gaps and areas for future evaluation/research. Data from the Toxicology in the 21st Century high throughput screening initiative ("Tox21") was also considered during the meeting. Experts used the data, primarily derived from Phase I of ToxCast™, to help evaluate biological plausibility as well as to develop testable predictions of which chemicals might perturb biological processes related to diabetes and obesity. Experts were also asked to suggest relevant assay targets that could be included in Tox21 in the future to better screen for perturbations of biological processes involved in diabetes and obesity.

Obesity is a major risk factor for metabolic syndrome and subsequent type 2 diabetes. In the context of this workshop, there was no clear or consistent distinction between these health outcomes, and they were considered collectively across the various chemicals or chemical
classes considered. For some exposures, such as maternal smoking/nicotine, organotins and bisphenol A, obesity has been studied as a primary health outcome. For arsenic and POPs the focus was on diabetes, and obesity was considered as a potential confounding or modifying factor.

A PubMed search strategy was developed to identify studies for xenobiotic exposures related to diabetes and obesity using both a MeSH-based and a keyword strategy (see Supplemental Material). The keyword search identifies “new” articles that were not yet indexed in PubMed. Additional details about the articles considered relevant from this search and those that were “hand collected” during the course of preparing the background documents will be presented in subsequent publications dealing with specific chemicals.

Findings from all the human studies were summarized in an Excel file. This document can be used in conjunction with a new graphical display program referred to as the Metadata Viewer, a tool to help the research community search the existing epidemiological studies. In brief, the graphing program allows the user to structure queries to look at main findings for a variety of variables or combination of variables (e.g., chemical class, specific chemical, chemical by health outcome, etc.). Data can also be grouped or sorted in order to conduct more detailed assessments of patterns of findings. The presentation format for human studies is a “forest plot” graphical display as shown in Figures 1-3. The input data file for the diabetes/obesity workshop contains almost 800 main findings. This software program was used in the workshop to visually display data but was not used to conduct quantitative meta-analyses. The graphing program,
accompanying data file, and instructions for use are publically accessible at
http://DNTP.niehs.nih.gov/tools/MetaDataViewer/.

MAJOR FINDINGS

Maternal smoking and nicotine

The strongest conclusion from the workshop was that nicotine likely acts as a developmental obesogen in humans. This conclusion was based on the very consistent pattern of overweight/obesity observed in epidemiology studies of children of mothers who smoked during pregnancy (Figure 1) and was supported by findings from laboratory animals exposed to nicotine during prenatal development. Crude and adjusted odd ratios (ORs) were similar in the epidemiological studies suggesting that social and behavioral differences between smokers and non-smokers are not likely to account for the observed differences in overweight risk (Oken et al. 2008). Two recent meta-analyses used funnel plot methods to ascertain publication bias and concluded there was some evidence for publication bias, but not enough to negate the overall conclusion of increased risk (Ino 2010; Oken et al. 2008). The metabolic changes reported in the animal studies recapitulated “to a large extent” those seen in children of mothers who smoke.

The breakout group recognized that other components in cigarette smoke may also be contributing to the association between maternal smoking and childhood overweight/obesity; however the studies of nicotine in experimental animals provided compelling evidence that nicotine alone was the causal agent.

Arsenic
The workgroup that evaluated this literature concluded there was suggestive evidence for an association of diabetes with living in regions with relatively high environmental arsenic exposures (> 150 ppb) in drinking water (Figure 2). However, additional research is needed to determine whether the strength of the association establishes a contributory or causal relationship. The current literature was considered to provide “insufficient” evidence for an association with diabetes and arsenic in lower exposure areas (<150 ppb) in drinking water, although recent studies with better measures of exposure and outcome provided increased evidence for an association.

The literature on arsenic and diabetes in experimental animals was judged inconclusive, but findings from newer animal studies provide findings that are consistent with the human results. The body of existing studies is highly diverse, with considerable variation in the duration of treatment (one day to two years), routes of administration, and dose levels used in the studies. Most of the studies treated animals with sodium arsenite, As(III), or arsenic trioxide, but other arsenicals have also been studied (Aguilar et al. 1997; Arnold et al. 2003; Hill et al. 2009; Paul et al. 2008). The studies also vary in experimental design and model systems used to assess endpoints relevant to diabetes as a health effect. Most of the studies were not designed to examine the diabetogenic effects of chronic arsenic exposure. Although the literature as a whole was judged inconclusive, findings from recent studies that were designed to focus more specifically on diabetes appear consistent with those human studies that link arsenic exposure to diabetes. Supportive findings include impaired glucose tolerance in studies with mice or rats treated with As(III) for periods of several months at drinking water concentrations ranging from 5 to 50 ppm (Cobo and Castineira 1997; Paul et al. 2008; Paul et al. 2007; Wang et al. 2009).
Measures of insulin regulation may also be affected, i.e., increased HOMA-IR, by oral gavage treatment of Wistar rats with As(III) at a dose of 3.4 mg/kg bw/day for 90 days (Izquierdo-Vega et al. 2006) or treatment of pregnant female LM/ Bc/Fnn mice with 9.6 mg/kg As(V) by ip injection on gestational days 7.5 and 8.5 (Hill et al. 2009).

Most in vitro or mechanistic studies were not designed specifically to study the diabetogenic or adipogenic effects of arsenic. Nevertheless, these studies suggest several pathways by which arsenic could influence pancreatic β-cell function and insulin sensitivity, including oxidative stress, effects on glucose uptake and transport, gluconeogenesis, adipocyte differentiation, and \( \text{Ca}^{2+} \) signalling [reviewed in Tseng (2004), Diaz-Villasenor et al. (2007; 2008), Druwe (2010)].

Studies suggest that arsenic may exert negative effects on β-cell function \textit{in vitro} through several mechanisms depending on the concentration tested (Fu et al. 2010).

\textbf{Epidemiological studies of Persistent Organic Pollutants (POPs) and Diabetes}

Persistent organic pollutants (POPs) consists of broad classes organohalides (i.e., organochlorines, organofluorines, and organobromines). The POPs literature related to diabetes is quite varied in quality and complexity. It consists of almost 100 epidemiological studies that report ~500 findings relating to pathways and signs of diabetes or metabolic syndrome. Often results for multiple POPs are reported in the same study. The workgroup developed a rating for each of these studies based on study design and the strategy used to assess exposure and the health outcome. Studies were considered less useful if the diagnoses of diabetes came from death certificates, if diabetes was self-reported, if exposure was self-reported, or if exposure was not clearly measured. The breakout group then used the Metadata Viewer program to assess patterns...
of association between various chemicals or chemical classes and diabetes (Boyles et al. in press).

The workgroup concluded that there is evidence for a positive association of diabetes with certain organochlorine POPs based on collected analyses of cross-sectional, prospective/retrospective cohort, and occupational exposure studies. Initial data mining indicates strongest correlations of diabetes with trans-nonachlor, DDE/DDT, and dioxins/dioxin-like chemicals including PCBs (see Figure 3 for PCB findings). In no case was the data considered sufficient to establish causality. The very strong exposure correlations among some POPs (correlation coefficients of 0.50-0.90) make it difficult to identify individual POPs as potential causal agents.

Organotins and Phthalates (PPAR Activators)

Organotins and phthalates were considered together in a breakout group session because these classes both interact with peroxisome proliferator-activated receptors (PPARs). The PPARs are intimately involved in the regulation of adipocyte differentiation, production of adipokines (immunomodulatory proteins secreted by adipose tissue), metabolic syndrome, and insulin sensitivity (Janesick A 2011; Kahn and McGraw 2010; Li et al. 2011; Wang 2010). In addition, there is the potential for co-exposures to these two chemical classes as they have a common use as plasticizers in polyvinylchloride (PVC) plastics. The extent and magnitude of exposure is assumed to be higher for phthalates but exposure to organotins is not well characterized.
The pattern of stimulatory activity varies for specific PPAR receptor subtypes between the organotins (primarily TBT) and individual phthalates with the organotins appearing to have a stronger mechanistic profile for inducing “obesogenic” effects. The organotins are agonists for PPARγ as well as RXRα, two receptors known to have positive effects on adipocyte differentiation in vitro when activated (Grun et al. 2006; 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). Because PPARγ and RXRα heterodimerize, these compounds will stimulate both parts of the complex.

The phthalates are less potent activators of PPARγ compared to organotins with agonist activity occurring at a 1000x higher concentrations (~10-100 µM versus ~10-100 nM) and they have not been identified as agonists for RXRα. In contrast, the phthalates are more potent agonists for PPARα compared to PPARγ. In rodent models, PPARα appears to mediate high dose di(2-ethylhexyl) phthalate (DEHP)-induced body weight loss, and its role in regulating adipogenesis is less clear. The organotins are not considered activators of PPARα (personal communication with Bruce Blumberg, November 28, 2010).

Organotins

There are no epidemiological studies of organotin exposure and obesity or diabetes. There are poisoning incident reports, mostly in workers involved in applying the compounds for pesticide use, that describe incidents of hyperglycemia and/or glycosuria [(Colosio et al. 1991; Manzo et al. 1981), reviewed in NIOSH Criteria Document for Organotin Compounds (1976)]. Recent animal and mechanistic studies report stimulatory effects of TBT on adipocyte differentiation (in
vitro and in vivo) and increased amount of fat tissue (i.e., larger epididymal fat pads) 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 [(Kirchner et al. 2010). Although the organotins “obesogen” literature is relatively new, with few studies, the quality of the existing studies was considered high by the breakout group.

Phthalates

Three cross-sectional human studies of exposure to phthalates were discussed by the breakout group (Boas et al. 2010; Hatch et al. 2008; Stahlhut et al. 2007). These studies report some positive associations but did not provide sufficient evidence to conclude there is an association with diabetes or obesity. Rather, the epidemiology studies were considered exploratory with preliminary data suggesting the possibility of gender differences in associations, and that different phthalates may have different activities. In these studies the urinary phthalate metabolite mono-ethyl phthalate (MEP) was most often associated with higher BMI (Hatch et al. 2008), waist circumference (Stahlhut et al. 2007), or HOMA (Stahlhut et al. 2007). This observation was interesting because MEP is generally considered inactive with respect to the anti-androgen effects of phthalates. Mono-2-ethylhexyl phthalate (MEHP) was associated with decreased BMI in females older than 12 years (Hatch et al. 2008).

Understanding differences in activity for PPARα between humans and rodents is important with respect to understanding potential effects of phthalates on body weight and metabolic endpoints.
Animals treated with relatively high doses of phthalates, such as DEHP, typically display decreased body weight and fat mass (Itsuki-Yoneda et al. 2007; Sakurai et al. 1978) and these effects appear to be largely mediated via the PPARα agonist activities of DEHP metabolites (Feige et al. 2010; Martinelli et al. 2010). The effects on decreased body weight and fat mass were present in wild type mice, but not PPARα knockout mice, demonstrating the importance of PPARα in regulating the fat loss (Feige et al. 2010). However, Feige et al. (2010) also assessed the effects of DEHP in genetically modified mice in which the normal mouse PPARα gene was replaced with the human gene. In the humanized model, mice treated with DEHP gained more weight and had an increase in epididymal white adipose mass compared to the wild-type animals. While PPARγ in rodents and humans acts similarly, PPARα activity in the rodent model is stronger compared to humans and may mask effects mediated through PPARγ.

**Bisphenol A (BPA)**

Overall, this breakout group concluded that the existing data, primarily based on animal and *in vitro* studies, are suggestive of an effect of BPA on glucose homeostasis, inulin release and cellular signalling in pancreatic β-cells, and adipogenesis (Alonso-Magdalena et al. 2010; Miyawaki et al. 2007; Ryan et al. 2010; Somm et al. 2009). The existing human data on diabetes (Lang et al. 2008; Melzer et al. 2010) or as a developmental obesogen (Padmanabhan et al. 2008; Wolff et al. 2008; Wolff et al. 2007) were considered too few in number and inconsistent to draw meaningful conclusions.

Reaching clearer conclusions on “obesity” from the existing animal data was problematic. Although many studies report body weight gain following developmental exposure (with
generally inconsistent patterns found) the workgroup emphasized that body weight is not considered a good measure of obesity in rodents and only a few studies have assessed obesity with the preferred metrics, e.g., fat mass, fat pad weight, and cellularity of adipocytes.

There is inconsistency in the in vivo findings that may relate to differences in experimental design, e.g., diet, route of administration, and species/strain. Understanding the basis for these inconsistencies was considered a research priority. The group also noted that the mechanisms of BPA action are not fully understood but that it acts as more than an estrogen receptor agonist. A number of in vitro findings suggest interactions with other receptor systems involved in metabolic regulation (Wetherill et al. 2007), including anti-androgen effects at low concentrations and high binding affinity for estrogen-related receptor-γ (ERRγ) (Takayanagi et al. 2006).

**Pesticides**

The pesticide breakout group concluded the epidemiological, animal, and mechanistic data support the biologically plausibility that exposure to multiple classes of pesticides (primarily insecticides) may affect risk factors for diabetes and obesity, although many significant data gaps remain. Some pesticide active ingredients, insecticides in particular, impact neurotransmitter and/or ion channel systems that are also involved in regulating pancreatic function, including acetylcholine (e.g., organophosphate, carbamate, neo-nicotinoids), sodium channels (e.g., pyrethroids), GABA (e.g., organochlorine), catecholamine (e.g., amidine/formamidine), and mitochondrial function (e.g., rotenone). This raises the possibility that these compounds might affect glucose homeostasis, at least at dose levels where they are effective as pesticides (Franklin
and Wollheim 2004; Satin and Kinard 1998). Much less research has focused on whether
pesticides have activities that might affect adiposity or other risk factors for metabolic syndrome.

Case reports of hyperglycemia have been reported following poisoning incidents with a variety
of pesticides, perhaps best documented for organophosphates (Agency for Toxic Substances and
Disease Registry (ATSDR) 1997; Sungur and Guven 2001) and the formamidine insecticide
amitraz (Caksen et al. 2003; Elinav et al. 2005; Ertekin et al. 2002; Kennel et al. 1996; Ulukaya
et al. 2001; Yilmaz and Yildizdas 2003). Type 1 diabetes is a recognized complication following
accidental poisoning with Vacor, a rodenticide chemically similar to streptozotocin, containing
~2% N-3-pyridylmethyl N'-p-nitrophenyl urea (PNU), and removed from the market in 1979
Pont et al. 1979; Prosser and Karam 1978; Yoon 1990). With the exception of studies of
persistent organochlorine pesticides such as DDT/DDE or trans-nonachlor, there are very few
cohort studies for other pesticides and health conditions related to diabetes, metabolic syndrome,
or adiposity.

There is a literature reporting effects of intoxication with OP insecticides on blood glucose in
laboratory animals, generally finding hyperglycemia at high dose levels (Karami-Mohajeri and
Abdollahi 2010; Rahimi and Abdollahi 2007). Recently, the focus of investigations has shifted
towards studies designed to understand the consequences of developmental exposure to lower
doses of organophosphates and long-term health effects related to metabolic dysfunction,
diabetes, and obesity later in life (Adigun et al. 2010a; Adigun et al. 2010b, c; Icenogle et al.
2004; Lassiter et al. 2010; Lassiter et al. 2008; Levin et al. 2002; Roegge et al. 2008; Slotkin et
al. 2005; Slotkin et al. 2009) [reviewed in Slotkin (2010)]. The general findings are that early-life exposure to otherwise subtoxic levels of OPs results in subsequently-emerging pre-diabetes, abnormalities of lipid metabolism, and promotion of obesity in response to increased dietary fat.

Along with the primary literature cited in this section, the Toxicity Reference Database, or ToxRefDB, was also used as a resource (http://www.epa.gov/ncct/toxrefdb/). The current version of ToxRefDB contains data for pesticide registration purposes for 474 chemicals, primarily pesticide active ingredients. These data are not available in the peer-reviewed literature.

ToxRefDB was queried for chemicals that caused increased body weight (or body weight gain), increased blood glucose, and pancreatic effects including mass, adenomas, and non-neoplastic outcomes (atrophy, congestion, hyperplasia, hypertrophy, inflammation, fatty change, degeneration, cellular infiltration). Approximately 100 chemicals were identified as causing at least one of these effects (http://DNTP.niehs.nih.gov/go/36433, see Appendix B). It is interesting to note that six of the studies identified increased body weight as an outcome from treatment with several organophosphates, including two separate studies for fenthion, one conducted in rats and the other in mice (Table 1). Several other pesticides, sulfonylurea herbicides and imidazole fungicides, identified from the ToxRefDB search belong to the same general chemical class as agents either currently used to manage type 2 diabetes or being researched for their value as therapeutic agents. In the majority of cases the same pesticide active ingredients identified from the ToxRefBD search were also screened in ToxCast™. Preliminary analysis of these results suggests that some of these chemicals may be impacting biochemical or cellular targets that have not been identified in the peer-review literature but support the biological plausibility for how
they may exert effects on glucose homeostasis, insulin sensitivity, or adipocyte differentiation and function.

**Utilization of Tox21 high throughput screening to identify substances of potential interest**

Consideration of data from the Tox21 high throughput screening initiative played a prominent role in the workshop. Tox21 is a collaborative program between the EPA, NIEHS/DNTP, NIH Chemical Genomics Center (NCGC), and FDA designed to research, develop, validate and translate innovative chemical testing methods that characterize toxicity pathways ([http://DNTP.niehs.nih.gov/go/28213](http://DNTP.niehs.nih.gov/go/28213)). Data from Phase I of ToxCast™, EPA’s contribution to Tox21, was used to help determine the biological plausibility of reported effects as well as to identify other chemicals that may interact with relevant mechanistic targets but have not been assessed for effects related to diabetes or obesity. In general, the ToxCast™ data often aligned with mechanistic findings in the peer-reviewed literature. The organotin fentin was identified in ToxCast™ as a target for PPARγ at a relatively low concentration ([http://actor.epa.gov/actor/faces/ToxCastDB/Home.jsp](http://actor.epa.gov/actor/faces/ToxCastDB/Home.jsp), search for “fentin”). Amitraz, a formamidine insecticide, is a α2-adrenoceptor agonist (Chen and Hsu 1994; Hugnet et al. 1996; Smith et al. 1990) and this activity was identified in ToxCast™ ([http://actor.epa.gov/actor/faces/ToxCastDB/Home.jsp](http://actor.epa.gov/actor/faces/ToxCastDB/Home.jsp), search for “amitraz”).

Many of the pesticides identified from ToxRefDB™ as causing increased body weight, increased blood glucose, or pancreatic effects were also screened in Phase I of ToxCast™, providing a framework for considering potential mechanisms that may underlie the in vivo effects. In this respect, it is noteworthy that many pesticides have HTS “hits” that fall outside their classic
pesticide mechanism of action that may be relevant to biological process of diabetes and obesity, including PPARγ. It should be noted that those chemicals, or chemical classes, with the strongest associations in humans (i.e., trans-nonachlor, TCDD, DDE/DDT, PCBs, arsenic, nicotine) have not yet been tested in ToxCast™.

In brief, experts identified relevant HTS targets for several biological processes related to diabetes and obesity (insulin signalling in pancreatic beta cells, islet cell function, adipocyte differentiation, and feeding behaviour in *C. elegans*). Experts also suggested biological assay targets that could be added to Tox21 to improve ability to identify chemicals that may perturb metabolic processes. The 309 chemicals tested in Phase I of ToxCast™, primarily pesticide active ingredients, were then screened against these targets to identify a set of chemicals predicted to perturb these process and others predicted to have no effect. As a follow-up activity, the DNTP is initiating a targeted testing activity for a set of predicted “positives” and “negatives” using more physiologically-based *in vitro* model systems.

**CONCLUSIONS, RESEARCH RECOMMENDATIONS, AND NEXT STEPS**

Overall, the workshop review of the existing literature supports the plausibility of an “obesogen” hypothesis, as well as a linkage to type 2 diabetes, of varying degrees, with exposures to certain chemical classes. A review of the literature indicates very little research has been directed towards understanding associations of exposures to xenobiotics with type 1 diabetes. This was considered a critical data gap. Many research questions remain and an important goal of this workshop was to identify data gaps to stimulate focused research to move the field forward. The research recommendations included suggestions for the most appropriate endpoints to evaluate in
human, animal and mechanistic studies of diabetes and obesity (Table 2 and Table 3).

Understanding more about the different phenotypes of obesity will require more sophisticated measurement methods. As shown in Figure 4, the distribution of adipose tissue can vary in individuals with the same BMI and waist circumference. Another series of recommendations was to elucidate the role(s) of effect modifiers, confounding factors and the role of the specific genetic contributions in humans and animal models used to study these diseases.

Many of the identified research gaps were not unique to the field of diabetes/obesity research. The workshop noted 1) deficiencies in data on human exposures to many of the chemicals examined, 2) the need for better biomarkers of exposure that may be related mechanistically to the disease endpoints, 3) the need for a better understanding of the basic biology of critical cells (i.e. adipocyte, beta-cell) functioning in health and disease, and 4) an appreciation of how the biology that controls body weight and metabolic set points change with life stage. The workshop specifically noted the need to appreciate and expect non-monotonic dose response relationships for environmental influences on obesity and diabetes, and recognized the enormous complexity inherent in the field. Finally, the workshop found the incorporation of high-throughput screening information from the Tox21 program to be an intriguing and useful way of improving our understanding of the similarities and differences in biological actions across classes of chemicals, and recommended many specific targets for further assay development to further enhance its utility.

NIEHS has already taken steps to address some of the research needs recognizing that some of this work will best be accomplished through the DNTP and other research through the NIEHS
Division of Extramural Research and Training (DERT) or Division of Intramural Research (DIR). Based in the results of this workshop and the data gaps noted DERT released a program announcement focused on improving our understanding of the role of environmental exposures in the development of obesity and diabetes (see program announcements PAR-11-170 and 171) ([http://www.niehs.nih.gov/funding/grants/announcements/announcements/index.cfm](http://www.niehs.nih.gov/funding/grants/announcements/announcements/index.cfm)). The announcement has one receipt date per year for the next three years. The DNTP is organizing further *in vitro* targeted testing of some of the predictions of chemical effects from the Tox21 screening program, and is specifically developing an analytical method to measure organotins in human blood since the essentially complete lack of exposure data to these compounds was considered a critical research need.

We hope this workshop will stimulate furthering research to better understand the public health impacts of environmental influences on the increasing international prevalence of diabetes, obesity and metabolic syndrome. We acknowledge the dedicated efforts of the workshop participants towards achieving this goal.
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**FIGURE AND TABLE LEGENDS**

**Figure 1.** Association between maternal smoking and overweight/obesity in offspring.

Abbreviations: Amer. Ind – American Indian; ALSPAC – Avon Longitudinal Study of Parents and Children; adjOR – adjusted odds ratio; BBC – British Birth Cohort; BMI – body mass index; CESAR – Central European Study on Air Pollution and Respiratory Health; CLASS – Children’s Lifestyle and School Performance study; CPP – Collaborative Perinatal Project, GDM – gestational diabetes mellitus, Gen R – Generation R study; NCDS – National Child Development Study; NLSY – National Longitudinal Survey of Youth, PedNSS – Pediatric Nutrition Surveillance System, WIC – Women, Infants, and Children program, RR – relative risk; wt. – weight; ref. – referent group

*Risk estimates for bracketed statistics, i.e., [crudeRR] calculated based on data presented in the paper using an open source epidemiology statistics programs, OpenEpi ([http://www.openepi.com/menu/openEpiMenu.htm](http://www.openepi.com/menu/openEpiMenu.htm))

**Figure 2.** Association between arsenic and diabetes in areas of relatively high exposures (>150 ppm drinking water).

Abbreviations: As – arsenic; adjOR – adjusted odds ratio; CEI – cumulative exposure index; CS – cross sectional; HEALS – Health Effects of Arsenic Longitudinal Study; OGTT – oral glucose tolerance test; PR – prevalence ratio; Pros – prospective; Retro – retrospective; RR – relative risk; SMR – standardized mortality ratio

*Risk estimates for bracketed statistics, i.e., [SMR] and [RR] were estimated by Navas-Acien et al. (2006)*
Figure 3. Association between PCBs and diabetes

Abbreviations: adjOR – adjusted odds ratio; CARDIA – Coronary Artery Risk Development in Young Adults; CC – case control; CS – cross sectional; NHANES – National Health and Nutrition Examination Survey; PBB – polybrominated biphenyl; Pros – prospective; WHILA – Women’s Health in the Lund Area

Figure 4. Imaging different types of obesity: Two subjects with the same BMI (32.0) and waist circumference (112 cm).

Table 1. Selected results from ToxRefDB search for chemicals that caused increased body weight (or body weight gain), increased glucose, or pancreatic effects

The complete list can be found at [http://ntp.niehs.nih.gov/ntp/ohat/diabetesobesity/Wkshp/PesticidesDraftLiteratureReviewV3formatted.pdf](http://ntp.niehs.nih.gov/ntp/ohat/diabetesobesity/Wkshp/PesticidesDraftLiteratureReviewV3formatted.pdf) (see Table 9).

Table 2. Research recommendations for health outcome assessment measures

Table 3. Diagnostic criteria, signs/symptoms, and risk factors for diabetes and obesity (BMJ)