COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Scoping paper on the obesogen hypothesis.

Obesogens

1. Over the last two decades, the incidence of obesity and associated metabolic syndrome diseases has risen dramatically. Increased caloric intake and decreased physical activity are believed to represent the root causes of this dramatic rise. However, more recently some researchers have suggested the possible involvement of environmental obesogens, xenobiotic chemicals that can disrupt the normal developmental and homeostatic controls over adipogenesis and energy balance.

2. In 2002, Paula Baillie-Hamilton proposed a hypothesis linking exposure to chemicals with obesity. This was based on observations that whilst exposure to high concentrations of some chemicals caused weight loss, exposure to low concentrations led to weight gain in adult animals. In 2006 the effects of chemicals on weight gain were reformulated as the “obesogen hypothesis”, a term coined by the American developmental biologist Bruce Blumberg. The obesogen hypothesis proposed that developmental exposure to endocrine disrupting chemicals (EDC) can induce permanent physiological changes. EDC exposure elicits epigenetic alterations in gene expression that reprograms the fate of mesenchymal stem cells, predisposing them to become fat cells. According to the theory, exposed animals develop more and larger fat cells, despite normal diet and exercise which leads to weight gain and obesity over time.

3. The environmental obesogen hypothesis predicts that inappropriate receptor activation by certain chemicals will lead directly to adipocyte differentiation and a predisposition to obesity and/or will sensitize exposed individuals to obesity and related metabolic disorders under the influence of the typical high-calorie, high-fat Western diet. Obesogenic chemicals could act in different ways to disrupt adipose tissue biology. Three main mechanisms of action have been proposed;

- alterations in the action of metabolic sensors in which obesogens mimic metabolic ligands acting to either block or up-regulate hormone receptors;

- dysregulation of sex steroid synthesis, in which they alter the ratio of sex hormones leading to changes in their control of lipid balance; and,

- changes in the central integration of energy balance including the regulation of appetite and satiety in the brain and the reprogramming of metabolic set-points.

4. The obesogen theory has been around for several years but as yet there appears to be limited data and no clear hypothesis. Further research to investigate the obesogen theory is on-going internationally. The report of the 2011 state-of-the-science workshop entitled “Role of Environmental Chemicals in the Development of Diabetes and Obesity” organised by the National Institute of Environmental Health Sciences (NIEHS) Division of the National Toxicology Program (NTP) to look at ways of investigating the theory was published in 2012. Members agreed in the COT 2012 Horizon scanning that this would provide an opportunity to evaluate the current evidence for the theory and the proposed research strategies. This paper summarises the NTP workshop and several other recent reviews of the obesogen theory and possible mechanisms. The detailed bibliographies and supporting reference for the evidence summarised in this paper can be found in the original reports which are appended. The paper seeks Members views on specific points in the papers presented, current evidence for the obesogen theory itself and whether more detailed evaluation of the theory should be a priority or is premature at this time.

NTP Workshop - Thayer *et al*. (2012); Role of Environmental Chemicals in Diabetes and Obesity: A National Toxicology Program Workshop Review

5. The NTP workshop was intended to evaluate the literature for evidence of associations between certain chemicals and risk of diabetes and/or obesity through an expert elicitation exercise involving more than 50 scientists including endocrinologists, toxicologists, epidemiologists, bioinformaticists and experts in the pathobiology of diabetes and obesity. They were asked to evaluate the current literature for consistency and biological plausibility and provide advice on developing a research programme. The specific environmental exposures evaluated were arsenic, maternal smoking during pregnancy/nicotine, organic tin compounds (“organotins”), phthalates, bisphenol A (BPA), pesticides, and various persistent organic pollutants (POPs). Since obesity is a major risk factor for metabolic syndrome and type 2 diabetes, all three outcomes were reviewed in relation to the environmental exposures evaluated, although the primary focus and context varied for specific exposures. The full published report is included at Annex 1.

6. The workshop comprised an introductory plenary session followed by a series of breakout group discussions and further plenary sessions to disseminate and discuss the outputs from the breakout group deliberations. Papers summarising the data were distributed before the workshop to provide a focus for the discussions. The breakout groups were not required to reach consensus on the questions and their reports reflected the range of opinions

expressed. For the individual chemicals or chemical classes, participants were asked to

a) evaluate the strengths, weaknesses, consistency and biological plausibility of findings reported in humans and experimental animals;
b) identify the most useful and relevant end points in experimental animals, *in vitro* models and screening systems to assess these diseases; and
c) identify data gaps and areas for future evaluation/research.

Data from the Toxicology in the 21st Century (Tox21) High-Throughput Screening (HTS) Initiative were also considered. Data (primarily derived from phase I of the U.S. Environmental Protection Agency (EPA) ToxCast™) were used to help evaluate biological plausibility and 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 these biological processes.

7. For producing the summary papers, a PubMed search strategy was developed to identify studies of xenobiotic exposures related to diabetes and obesity using both a MeSH (Medical Subject Headings)-based strategy and a keyword strategy. A complete list of MeSH and keyword search terms was made available at http://dx.doi.org/10.1289/ehp.1104597]. The keyword search was included to identify newer articles that were not yet MeSH indexed in PubMed at the time of the search.

8. Data on the main findings from studies considered relevant were extracted by NTP staff in the Office of Health Assessment and Translation (OHAT) based on the following strategy. For studies that did not report a significant association between the exposure and a health outcome, data extracted were based on the highest exposure group compared with the referent group (e.g. fourth quartile vs. first quartile). When a study reported a significant association between an exposure and a health outcome, the data extracted for monotonic exposure–response relationships were based on the lowest exposure group where a statistically significant association was observed (e.g., third quartile vs. first quartile). For non-monotonic associations main findings were identified on a case-by-case basis and data extracted included consideration of any statistical trend analyses that might have been conducted, consistency of the overall pattern across exposure groups, and/or consideration of the author's interpretation of the biological significance of the non-monotonic finding. The extracted data were stored in an Excel file that could be used with a new graphical display software program called Meta Data Viewer. The graphing program allows users to sort, group, or filter studies according to exposures, health outcomes, and other characteristics and can present the main findings using a "forest plot" graphical display. The input data file for the diabetes/obesity workshop contains approximately 870 main findings from > 200 human studies. This software program was used during 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 publicly accessible http://ntp.niehs.nih.gov/go/tools_metadataviewer .

Conclusions on environmental exposures evaluated in the workshop.

Arsenic.

9. The existing human data were considered to provide limited to sufficient evidence in support of an association between arsenic and diabetes (type not specified) in populations with high exposure levels, i.e. regions in Taiwan and Bangladesh with historical problems with arsenic contamination of drinking water. Although the majority considered the evidence sufficient for an association, additional research was required to determine whether a causal relationship exists. The current evidence for lower-exposure areas (< 150 ppb arsenic in drinking water) was insufficient to establish an association with diabetes, although more recent studies with better measures of exposure and outcome were considered more suggestive of an association. The literature on arsenic and diabetes in experimental animals was inconclusive. The studies were highly diverse with considerable variation in the duration of treatment (1 day to 2 years), routes of administration, dose levels, experimental design, arsenicals tested and model systems used to assess end points relevant to diabetes. There was some evidence from studies designed to focus more specifically on glucose homeostasis which appeared consistent with human studies linking arsenic exposure to diabetes. Available *in vitro* or mechanistic studies were not generally designed to study the diabetogenic or adipogenic effects of arsenic. Nevertheless, participants considered these studies suggested several pathways by which arsenic could influence pancreatic β-cell function and insulin sensitivity, including oxidative stress and effects on glucose uptake and transport, gluconeogenesis, adipocyte differentiation, and Ca^{2+} signalling). Arsenic may exert adverse effects on β-cell function *in vitro* through several mechanisms, depending on the concentration tested.

Maternal smoking during pregnancy/nicotine.

10. The strongest conclusion from the workshop was that nicotine probably acts as a developmental obesogen in humans based on the very consistent pattern of overweight and/or obesity observed in epidemiology studies of children of mothers who smoked during pregnancy supported by findings from laboratory animals exposed to nicotine during prenatal development. The similarity of crude and adjusted odds ratios (ORs) in the individual epidemiological studies was seen as evidence that the social and behavioural characteristics included in models did not account for the observed. Although there was some evidence for publication bias reported in two recent meta-analyses, this was considered insufficient to negate the overall conclusion of an increased risk. The studies of nicotine in experimental animals provided compelling evidence to the breakout group that nicotine alone was likely to be the causal agent, however they could not exclude the possibility that other components in cigarette smoke may also be contributing to the association.

Organic tin compounds ("organotins") and phthalates,

11. Organotins and phthalates were considered together because these compounds both interact with PPARs. The PPARs are intimately involved in the regulation of adipocyte differentiation, production of adipokines, insulin responsiveness, and other biological processes related to glucose and lipid regulation. In addition, both are commonly used as plasticizers in PVC (polyvinylchloride) plastics so co-exposures to these two chemical classes occur. Exposure to phthalates was assumed to be higher than to organotins.

12. The pattern of specific PPAR receptor subtype interactions of organotins [primarily tributyltin (TBT)] and individual phthalates differs. The organotins are potent agonists for PPARγ and retinoid X receptor-α (RXRα), when activated these two receptors promote adipocyte differentiation *in vitro*. Moreover PPARγ and RXRα heterodimerize, as organotins interact with both parts of the heterodimer complex they could be greater than expected. Phthalates are 1000-fold less potent agonists of PPARγ than are organotins and have not been identified as agonists for RXRα. In contrast to the organotins, the phthalates are more potent agonists for PPARα than for PPARγ. There seems to be a stronger mechanistic rationale by which organotins could have the potential to induce "obesogenic" effects.

13. No epidemiological studies of organotin exposure and obesity or diabetes were identified during the literature search. Incidents of hyperglycemia and/or glycosuria have been described in poisoning incident reports, mostly in workers involved in applying the compounds for pesticidal use. 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 and increased lipid accumulation in adipocytes Although limited in number, the quality of the existing experimental studies was considered high.

14. Three cross-sectional human studies of exposure to phthalates were discussed which reported some positive associations but did not provide sufficient evidence to conclude there was an association with diabetes or obesity. There are differences in PPARα activity between humans and rodents with two to three fold higher concentrations of phthalate monoester required to activate human PPARα compared to mouse PPARα, and lower maximum-fold induction for human PPARα). PPARγ activity is similar in rodents and humans, but stronger PPARα activity in mice compared with humans may mask effects mediated through PPARγ.

Bisphenol A (BPA),

15. Overall, primarily based on animal and *in vitro* studies, existing data suggested that BPA could have effects on glucose homeostasis, insulin release, cellular signaling in pancreatic β cells and adipogenesis. The available human data on BPA and diabetes (Lang et al. 2008; Melzer et al.

2010) were considered too limited to draw meaningful conclusions. Similarly, data from a single pilot study were insufficient to evaluate BPA as a potential risk factor for childhood obesity.

16. It was not possible to conclude on BPA and obesity from the existing animal data. Although several studies reported body weight gain after developmental exposure, the overall pattern across studies was inconsistent. However, concerns were expressed that body weight was not a good measure of obesity in rodents and the number of studies using the preferred metrics such as fat mass, fat pad weight, and cell adipose tissue cellularity was very limited. The inconsistency in the *in vivo* findings may be attributable to experimental design such as differences in diet, route of administration and species/strain. Understanding the basis for these inconsistencies was considered a research priority.

Pesticides.

17. It was concluded that the epidemiological, animal and mechanistic data supported the biological plausibility of exposure to multiple classes of pesticides affecting risk factors for diabetes and obesity, although many significant data gaps remain. Some active ingredients affected neurotransmitter and/or ion channel systems involved in regulating pancreatic function. At dose levels where they are effective as pesticides these compounds might affect glucose homeostasis. Much less research has focused on whether pesticides might affect adiposity or other metabolic syndrome components.

18. Case reports of hyperglycemia have been reported after poisoning incidents with a variety of pesticides. Type 1 diabetes is a recognized complication after accidental poisoning with the banned rodenticide Vacor, which is structurally similar to streptozotocin (a compound widely used to induce experimental diabetes in animals). With the exception of persistent organochlorine pesticides such as DDT/DDE or *trans*-nonachlor, there are very few cohort studies of other pesticides and health conditions related to diabetes, metabolic syndrome or adiposity.

19. There have been numerous reports of organophosphate insecticides affecting blood glucose in laboratory animals, generally resulting in hyperglycemia at high dose levels. Recently, studies have investigated developmental exposure to lower doses of organophosphates and possible long-term effects of these exposures on metabolic dysfunction, diabetes and obesity later in life. Early-life exposure to otherwise sub-toxic levels of organophosphates resulted in pre-diabetes, abnormalities of lipid metabolism and promotion of obesity in response to increased dietary fat.

20. The EPA Toxicity Reference Database (ToxRefDB) contains detailed study and effect information on 474 chemicals, principally data-rich pesticide active ingredients. Many of these studies were conducted for regulatory purposes and were 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 effects on the pancreas, including changes in mass, adenomas, atrophy, congestion, hyperplasia, hypertrophy, inflammation, fatty change, degeneration, and cellular infiltration. Approximately 100 chemicals caused at least one of these effects. Six of the studies identified increased body weight as a result of treatment with several organophosphates, including two separate studies for fenthion, one conducted in rats and the other in mice. Several sulfonylurea herbicides and imidazole fungicides were also identified by the ToxRefDB search. These pesticides belong to the same general chemical class as agents used to manage type 2 diabetes or being investigated as potential therapeutic agents.

Persistent organic pollutants (POPs)

21. The POP literature related to diabetes and other metabolic disorders is complex, consisting of approximately 75 epidemiological studies that report hundreds of findings relating to diabetes, altered glucose homeostasis, insulin resistance or metabolic syndrome. Often results for multiple POPs are reported in the same study. Because of time constraints, the workshop focused on diabetes outcomes. A quality rating was developed for each study based primarily on the methods used to classify or measure exposure and how diabetes status was ascertained. Studies received a lower rating if the diagnoses of diabetes came from death certificates or was self-reported, if exposure was self-reported or not clearly measured. The Meta Data Viewer program was then used to assess patterns of association between various POPs chemicals or chemical classes and diabetes.

22. The group concluded that there was evidence for a positive association of diabetes with certain organochlorine POPs. Initial data mining indicated the strongest associations were with *trans*-nonachlor, DDT (dichlorodiphenyltrichloroethane)/DDE (dichlorodiphenyldichloroethylene)/DDD (dichlorochlorophenylethane), dioxins and dioxin-like chemicals including polychlorinated biphenyls (PCBs). In no case was the body of data considered sufficient to establish causality.

Conclusions and research recommendations.

23. Overall, the workshop review of the existing literature supported the plausibility of the “obesogen” hypothesis as well as linkages between type 2 diabetes and exposures to certain chemical classes. A review of the literature indicated very little research on understanding associations between environmental exposures and type 1 diabetes. This was considered a critical data gap. Many research questions remained, 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 end points to evaluate in human, animal, and mechanistic studies of diabetes and obesity (Tables 2–4 of Annex 1). The importance of using clinically accepted measures of diabetes and overweight/obesity in the

epidemiological studies was stressed. Understanding more about the different phenotypes of obesity will require more sophisticated measurement methods because the distribution of adipose tissue can vary among individuals with the same BMI and waist circumference. Another series of recommendations was to elucidate the role(s) of effect modifiers, confounding factors, and specific genetic contributions in humans and animal models used to study these diseases (Table 3).

24. Many of the research gaps were not unique to the field of diabetes/obesity research. The workshop noted

a) deficiencies in data on human exposures to many of the chemicals examined,

b) the need for better biomarkers of exposure that may be related mechanistically to the disease end points,

c) the need for a better understanding of the basic biology of adipocytes, β cells, and neural circuits that regulate feeding behavior in healthy and disease states, and

d) the need for an appreciation of how the biology that controls body weight and metabolic set points changes with life stage.

A number of the breakout groups noted the need to consider non-monotonic dose–response relationships for environmental influences on obesity and diabetes. Also, there was a need to consider co-exposures between environmental chemicals and consumption of high-calorie, high-carbohydrate, and/or high-fat diets. Finally, workshop participants found the incorporation of HTS 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.

Chem Trust report - Porta and Lee (2012); Review Of The Science Linking Chemical Exposures To The Human Risk Of Obesity And Diabetes.

25. In March 2012 Chem Trust published a review it had commissioned on the links between chemical exposures and obesity and diabetes. A copy of the report is appended at Annex 2.

26. A number of scientific papers have been published on the potential mechanisms by which obesogenic chemicals might cause obesity. A detailed analysis of biological mechanisms was outside the scope of the Chem Trust report but a brief summary of selected findings from recent cell and animal studies was included.

27. The report defines the obesogen hypothesis as essentially that exposure to exogenous chemicals disrupts adipogenesis and the normal regulation of lipids (through homeostasis and metabolism) ultimately resulting in obesity. According to the report a functional definition of obesogens would be chemicals that alter homeostatic metabolic set-points, disrupt appetite controls, perturb lipid homeostasis to promote adipocyte hypertrophy,

stimulate adipogenic pathways that enhance adipocyte hyperplasia or otherwise alter adipocyte differentiation during development.

28. The report suggests these chemicals could be considered EDCs since proposed pathways include inappropriate modulation of nuclear receptor function. As such embryonic, fetal and infantile stages could be more vulnerable to disruption from relatively low doses. Another possibility highlighted is that it could be an example of fetal origins of adult disease in which the risk of developing chronic diseases in adulthood is highly influenced by environmental factors acting early in life. An example being the observations that maternal protein deficiency or under-nutrition can limit fetal growth resulting in a low birth weight and a predisposition to obesity and insulin resistance in adulthood. The report suggests that since limiting fetal development by poor food availability during pregnancy is not likely to be widespread in the developed world, exposure to chemicals may be more relevant. It further suggests that effects on epigenetic processes might have a plausible role in the fetal origins of obesity and insulin resistance.

Evidence for links with obesity.

29. The possible candidate obesogens identified in the report have a wide range of different chemical and physical properties and the authors suggest that it is probable there are other as yet unrecognised chemicals. Therefore, they suggest discussing several common molecular mechanisms is more appropriate than focusing on specific chemicals. Three mechanisms are highlighted; binding to peroxisome proliferator-activated receptor (PPAR) γ; inappropriate activation of estrogen receptors (ER) and modulating the pregnane X receptor (PXR) and constitutive androstane receptor (CAR). The authors suggest multiple mechanisms may exist as not all chemicals that activate PPARγ are adipogenic or correlated with obesity in humans.

30. While the authors considered there was substantial laboratory evidence that chemicals can affect weight gain in animals which supports the hypothesis that EDCs promote or otherwise influence obesity, the evidence in humans was limited. Human studies can be divided into *in utero* exposures (prospective and mainly focused on persistent chemicals) and adult exposures (cross-sectional or prospective with persistent or non-persistent chemicals). Epidemiological studies on body weight and size following *in utero* exposure produced a range of results with negative to positive associations, depending on the chemical. In addition although some studies on *in utero* exposure to organochlorine pesticides had associations with future obesity, these findings were not replicated in other studies. The authors noted that observed positive associations tended to vary in different subgroups, particularly by gender. Similarly mixed results have been reported for associations of BMI and PCB exposure. Smoking in pregnancy has been associated with the likelihood of gaining excess weight in offspring as they grow up. The authors were unable to identify human studies on the effects of *in utero* exposure to non-persistent chemicals.

31. The authors noted that there had been an increase in the epidemiological literature following exposures during adulthood. Positive cross-sectional associations of serum concentrations of some POPs (such as DDT or dioxins) with adiposity were reported but again the association differed between genders. The interpretation of cross-sectional studies showing associations between serum concentrations of POPs and adiposity is further complicated because adiposity can modify the toxicokinetics of these chemicals. The literature is inconsistent and varies with individual POPs and their properties e.g. levels of the less chlorinated PCBs had positive associations with abdominal obesity in the elderly whereas highly chlorinated PCBs was inversely associated with abdominal obesity.

32. For non-persistent but ubiquitous compounds observations also differed by gender and age but interpretation of cross-sectional findings was further complicated as concentrations in serum or urine primarily reflected recent exposure. In addition with phthalates only some metabolites were positively associated with adiposity.

33. The major roles of diet and physical activity in obesity make testing hypotheses on the relationships between chemical exposures and obesity in humans particularly difficult. It may not be possible, even with sophisticated statistical adjustments, to completely eliminate the strong effects of diet and physical activity on obesity. This arises for several reasons; measurement errors in estimating calorie intake and physical activity in human beings, higher food consumption can lead to both obesity and increased body levels of chemicals, multiple exposures and possibility of mixture effects. It may be difficult or impossible to disentangle the specific contribution of each of many factors in epidemiological studies.

34. The authors concluded based on a significant and growing number of mechanistic studies and animal experiments, as well as on some clinical and epidemiological studies that chemicals in the environment may be partly responsible for the increasing occurrence of obesity in human populations. They consider the weight of evidence compelling, although it was difficult to prove such associations in human studies.

Evidence for links with diabetes.

35. A causal role for obesity in diabetes is well-established. Whilst evidence of a relationship between human environmental chemical levels and the risk of diabetes has existed for over 15 years, the authors considered the volume and strength of the evidence had become particularly persuasive since 2006. The authors identified the most interesting mechanistic and animal data suggesting that environmental chemicals play a role in the aetiology of diabetes. However they cautioned that diabetogenic agents can be defined in several ways such as chemicals causing obesity and insulin resistance as well as chemicals causing pancreatic β-cell dysfunction. These diabetogenic chemicals may induce effects in one or more categories. The

report discussed 3 chemicals (POPs, BPA and arsenic) in detail because these had substantial evidence from both experimental and human data

36. The report highlighted a number of potential mechanisms. It also suggested that certain chemicals may be simultaneously active on several biochemical pathways. Chemicals may act as EDCs, disrupting estrogen and generating a pregnancy-like metabolic state characterised by insulin resistance and hyperinsulinemia through acting on insulin-sensitive tissues and on β-cells. Other possible receptors included PXR (pregnane X receptors), CAR (constitutive androstane receptors), AhR (aryl hydrocarbon receptors), GR (glucocorticoid receptors) and PPAR (peroxisome proliferator activated receptors). Chemicals that bind to these PXR, CAR and the AhR receptors (including many hormone disruptors) can alter lipid and glucose metabolism. Llipophilic EDCs with properties that induce insulin resistance accumulate in adipose tissue with their release from adipocytes a potential link between obesity and insulin resistance.

Arsenic

37. An increased prevalence of diabetes (type not specified) has consistently been observed among residents in the high arsenic exposure areas in Taiwan and Bangladesh, showing a dose-response relationship with arsenic levels in drinking water. However, inconsistent findings have been reported from studies in low arsenic exposure areas. Systematic reviews of the literature suggest a possible role of high arsenic exposure (>500 ug/L) in diabetes.

38. *In vitro* and animal studies highlight that arsenic exposure could potentially increase the risk of diabetes through effects on the inhibition of insulin-dependent glucose uptake and insulin signalling, impairment of insulin secretion and transcription in pancreatic β-cells and modification of the expression of genes involved in insulin resistance. However, the concentrations used in most mechanistic experiments are much higher than concentrations seen in humans, and the observed effects may not be applicable to populations chronically exposed to arsenic via the environment.

BPA

39. There was some, albeit not entirely consistent, evidence from experimental studies of possible effects of BPA on glucose metabolism and insulin resistance, evidence in humans was limited. In a cross sectional analysis of the 2003-04 NHANES data from the US adult population, subjects reporting diabetes had higher concentrations of BPA in urine. However, a subsequent study using more recent 2005-06 NHANES data, the same researchers failed to find statistically significant associations with diabetes, although pooled estimates remained significant. The single urine sample from an individual in these studies mainly reflects recent exposure and was unlikely

to be a valid measurement of a subject's exposure history. The NHANES data does not distinguish between Type 1 and Type 2 diabetes.

POPs

40. The earliest evidence linking exposure to POPs with diabetes came from studies on 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD). There was a consistent finding of a slight increase in diabetes incidence among subjects with elevated serum TCDD concentrations, as well as abnormal glucose and/or insulin levels among subjects who were exposed to TCDD. Notably, a series of epidemiological studies on US Air Force veterans of Operation Ranch Hand involved in spraying defoliants during the Vietnam War led to a report by a committee of the National Academy of Sciences' Institute of Medicine, which concluded that there was suggestive evidence of an association between dioxin exposure and diabetes. The definition of diabetes in the original studies was verified history of diabetes mellitus by diagnosis or an oral glucose tolerance test of ≥11.1 mmol/L (200 mg/dL). Members will wish to be aware that the COT considered these studies in detail in 2001 when establishing the TDI for dioxins.

41. In the past few years, there have been cross-sectional studies and a few prospective studies on serum concentrations of several POPs and diabetes in the general population of different countries (mainly USA but some from Europe). The most compelling evidence was observed in a series of cross-sectional studies in the US using National Health and Examination Survey (NHANES) data. For example, when a summary measure of six POPs (those most commonly detected in this general population) was used, the adjusted odds ratios (the common estimate of the relative risk) for diabetes across quartiles of the summary measure were 1.0, 14, 14.7, and 38.3. In this study obesity was not associated with diabetes among people with very low levels of POPs; and diabetes itself was very rare even among people with BMI>=30kg/m^2. These findings suggested that a more significant role in the pathogenesis of diabetes was accumulation of POPs in the adipose tissue, rather than the adiposity itself.

42. One recent study performed multiple cross-sectional analyses associating 266 unique environmental factors with diabetes; it is an innovative Environmental-Wide Association Study (EWAS). The most significant associations were with organochlorine pesticides and PCBs. Recent prospective studies mostly confirmed the findings, although the specific kinds of POPs predicting diabetes and the shapes of the dose-response curves varied across studies.

Other chemicals

43. Besides organochlorine pesticides, there was also evidence of effects of pesticides such as organophosphates and carbamates on glucose and lipid metabolism through various mechanisms. Organophosphates and

carbamates exhibit these effects through inhibition of acetyl cholinesterase or by affecting target organs directly. These pesticides induce cellular oxidative stress via an effect on mitochondrial function which can disrupt glucose and lipid metabolism. Even though human evidence was scarce, two epidemiological studies among agricultural farmers have reported positive associations between use of some herbicides or insecticides and gestational diabetes or diabetes. Cigarette smoking was also linked to disturbance of glucose metabolism. Animal studies suggest maternal nicotine exposure may cause impaired glucose homeostasis in offspring as a result of both defective insulin secretion (caused by impaired pancreatic β-cell mass and function) and reduced peripheral insulin sensitivity.

Obesity papers

44. A series of 4 short papers were published in the Journal Obesity in June 2013, an editorial (Evidence for Obesogens: Interpretations and Next Steps by Gohlke and Allison) and three perspectives (The Perfect Storm for Obesity by Heindel and Schug; Obesogens and Obesity—an Alternative View? by Sharpe and Drake; An Integrated Approach to Assess the Role of Chemical Exposure in Obesity by Legler). These set out arguments for and against the hypothesis and ways to explore it but also highlight the complexity by showing how the same data can be used to support both arguments. These papers are included in Annex 3.

The Perfect Storm for Obesity by Heindel and Schug (2013);

45. The authors postulated that a subclass of endocrine disrupting chemicals (EDCs) by interfering with endocrine signalling can disrupt hormonally regulated metabolic processes, especially during early development. Through effects on metabolic tissues these may predispose some people to gain weight irrespective of efforts to limit caloric intake and increase physical activity. Alternatively links are being reported between chemical exposures early in life and later life onset of obsessive eating in obese individuals and other addictive behaviours. In the USA, the Presidential Task Force on Childhood Obesity and the National Institutes of Health Strategic Plan for Obesity Research both acknowledge that environmental exposures could play an underlying role in the development of metabolic diseases. The authors cited the NTP workshop (described earlier) as providing ‘‘real biological plausibility’’ for linkages between environmental exposures and type 2 diabetes and obesity based on many studies in both humans and animal models.

46. The authors considered early development as a highly orchestrated series of biochemical, physical, and organizational events which ensure proper growth only when tightly coordinated. This phase is characterised by a critical sensitiity to alterations in hormones resulting in changes in gene expression and protein levels, which persist as tissues and organs develop. Early life exposures (*in utero* and/or first few years of life) to environmental

perturbations (nutrition or environmental chemicals) can be directly associated with increased risk for many of today's most common diseases. Thus exposure at these times to obesogens could disrupt metabolic programming subsequently increasing risks of obesity later in life. For example, a single prenatal exposure in mice to tibutyltin (TBT) resulted in premature accumulation of adipose tissue. Developmental exposure to TBT altered the fate of mesenchymal stem cells, which to become fat cells rather than bone cells.

47. According to the authors nearly 20 chemicals following exposure during critical periods have been demonstrated to cause long-term weight gain due to disruption of normal hormone and neuronal signalling pathways. There could potentially be many more as close to 800 chemicals have reported EDC properties. In addition as obesogens are lipid-soluble, they may be more harmful to already overweight or obese individuals by being stored in fat cells where they can continue to disrupt tissue function and following release into the bloodstream during weight loss. These higher concentrations may increase the likelihood of regaining shed pounds by interfering with both the thyroid gland and individual cells' mitochondria, thereby affecting regulation of the body's metabolism and conversion of fuel into energy.

48. EDCs can disrupt the organization and function of dopaminergic pathways throughout the brain, resulting in a wide range of behavioural effects including elevated impulsivity. EDCs have been shown to trigger changes in the hypothalamus, which plays an important role in feeding behaviour.

49. Nutrition during development also plays an important role. Maternal diabetes was highly associated with above average birth weight and childhood obesity as were high pre-pregnancy body mass index and excessive weight gain during pregnancy. An above average birth weight can be an indicator for overweight or obesity in childhood and adulthood. Paradoxically, people born small for gestational age also have an increased risk of obesity, possibly because of rapid compensatory postnatal growth.

50. The authors considered that many disease patterns linked to poor nutrition have also been traced to maternal chemical exposure. This could indicate a common mechanism for chemical and nutritional stress that ultimately leads to obesity. They propose that the confluence of these factors during development (in utero and first few years of life), over nutrition, decreased activity and additional environmental exposures throughout life are driving the obesity epidemic throughout the world. More research is required into understanding how nutritional and environmental chemical exposures affect eating behaviour and the basic mechanisms underlying adipose tissue development and function.

Obesogens and Obesity—an Alternative View? by Sharpe and Drake (2013);

51. The prevalence of obesity more than doubled between 1980 and 2008 worldwide, so that by 2008, 10% of men and 14% of women in the world were obese (body mass index, BMI ≥ 30 kg/m^2) and at least 2.8 million people die each year as a result of being overweight or obese. Adiposity is highly heritable, with an estimated genetic contribution to BMI of 20-84%. However, although genome-wide association studies have identified many genomic regions associated with obesity, common forms of obesity are highly polygenic, with each variant contributing very small effects. The explanations most commonly advanced for the obesity epidemic are increased calorie intake and decreased energy expenditure; however in reality, many factors are likely to contribute, e.g. diet, exercise, economic, and psychosocial influences. These factors may have important interactions with some of the ‘‘obesity gene’’ variants.

52. A new hypothesis has emerged in recent years that environmental chemicals (termed obesogens) promote obesity. This is based on several lines of evidence, including numerous epidemiological studies in man. Further concerns have been raised by the increasing body of evidence for a lasting effect of the pre- and/or postnatal environment on the predisposition to later obesity. One feature of the implicated chemicals is that they are ubiquitous in our environment, so we are all exposed to greater or lesser degrees and, importantly, exposure has increased over recent years in parallel with the obesity epidemic. The strength and composition of the evidence is critical to assessing the hypothesis.

53. Studies have suggested that ‘‘environmental obesogens’’ can target cellular processes that are important in regulating metabolic function such as peroxisome proliferator (PPAR)-modulated pathways, adipogenesis, altered pancreatic *b* cell mass/function and altered hypothalamic circuits that modulate energy balance. Other *in vivo* studies have shown that exposure of rodents to tributyl tin or to bisphenol A can induce features of the metabolic syndrome including increase in weight/obesity. Whether human exposure is sufficient to induce such effects is unknown, as dietary exposure of rodents to more human-relevant doses of bisphenol A did not induce such effects. The biggest issue for the obesogen hypothesis is whether humans are exposed to sufficient amounts of either individual chemicals or mixtures of such chemicals to exert the types of biological effects shown *in vitro* or in experimental animal studies.

54. There are several cross-sectional epidemiological studies showing a significant association between concurrent urinary levels of bisphenol A and obesity or obesity-related disorders, e.g. type II diabetes although a more recent analysis using the same source material has questioned this. Other studies have shown a similar association of obesity with phthalate exposure. None of the ‘‘positive’’ studies show cause and effect. Since obesity is a long-term disorder, the association with concurrent exposure has obvious limitations. Nevertheless, such evidence cannot simply be dismissed, as it is clearly telling us something fundamental.

55. The authors propose that there may be an alternative explanation for these results. One of the most commonly advanced reasons for the increase in prevalence of obesity is consumption of a ‘‘Western’’ fast-food, high fat, energy-rich diet. If such a diet also determines our level of exposure to obesogens, then increased consumption of this diet would increase the risk of becoming obese at the same time as increasing exposure to environmental obesogens. Thus, by default the latter would become associated (non-causally) with obesity. There is reasonable, if indirect, supporting evidence for this: diet accounts for >95% of population level exposure to bisphenol A and phthalate, switching from a modern fast-food/processed/ packaged food diet to the same ingredients but freshly sourced and unpackaged lowered urinary levels of bisphenol A and phthalates markedly.

56. Arguably, more solid evidence supporting the obesogen hypothesis can be derived from prospective and cross-sectional studies that have shown positive associations between obesity and exposure to POPs. However, similar confounding with diet may also be present as lipophilic POPs accumulate in animal fat (and therefore in the food chain. Consequently, it would be reasonable to speculate that a modern ‘‘poor’’ high-fat diet resulted in increased exposure to POPs, although this needs to be formally tested. Other factors may also apply, for example POPs might increase thermogenesis and thus reduce weight gain, complicating interpretation of any association between POP exposure and bodyweight.

57. Poor diet and the imbalance between energy intake and expenditure are key factors in the obesity epidemic, which remains the major problem that must be addressed if the trend is to be reversed. Understanding whether there is any role for environmental obesogens is important. The epidemiological evidence in humans represents the only direct support, but if poor diet is also responsible for higher obesogen exposure, then this evidence is intrinsically confounded. Therefore, the authors consider that determining the role that poor diet plays in obesogen exposure is crucial. Only once this confounding is resolved will it be possible to show whether or not there is any substance to the obesogen hypothesis. Embarking on further epidemiological association studies before resolving this would be pointless.

An Integrated Approach to Assess the Role of Chemical Exposure in Obesity by Legler (2013)

58. OBELIX (“OBesogenic Endocrine disrupting chemicals: Linking prenatal eXposure to the development of obesity later in life”) is an ongoing multidisciplinary research project which focuses on assessing exposure to major classes of EDCs found in food. A series of five long-term animal studies are underway, in which mice are exposed to EDCs through the diet during gestation and lactation. Offspring are monitored up to adulthood. Doses in animal experiments reflect human exposure and levels are below no adverse effect levels for developmental toxicity. Epidemiological studies involve mother-child cohorts from four European countries in which perinatal EDC exposure is determined in cord blood and milk samples, and growth and

health status are followed up to 8 years. A more detailed explanation of the aims and design of the OBELIX project was published by Legler et al. (2011) and is included at Annex 5.

59. The compounds include dioxins and dioxin-like PCBs, non-dioxin-like PCBs, brominated flame retardants (BFRs), organochlorine pesticides, phthalates, perfluorinated alkyl acids and bisphenol A (BPA) Results from an as yet unpublished animal studies in OBELIX have revealed divergent sex-specific effects of perinatal exposure to BPA weeks after the exposure has ceased. These data indicate that gender stratification is essential when examining the role of chemical exposure in obesity development. The author noted that cross-sectional epidemiologic studies with phthalates have shown marked sex-specific differences in waist circumference and body mass index. Similarly studies from the Dutch Hunger Winter showed an association between prenatal famine and late life increase in BMI particularly in women.

60. EDC-mediated changes in epigenetic processes such as DNA methylation, histone modifications, and microRNAs are proposed to play a role in the developmental origins of obesity. In OBELIX, mechanistic studies focus on understanding the role of changes in DNA methylation after developmental EDC exposure. Studies in the murine 3T3-L1 preadipocyte cell line with a variety of EDCs resulted in enhanced differentiation of adipocytes, generally accompanied by global DNA hypomethylation and specific changes in gene expression and promoter methylation of PPARc. However the author acknowledged that confirmation that this *in vitro* model predicts effects in humans remains a daunting task. For some EDCs, such as tributyltin and BPA, adipogenic responses in 3T3-L1 cells correlate with obesogenic effects *in vivo* following prenatal exposure in rodents. However, considerable differences existed between the 3T3-L1 and human adipocyte transcriptome and epigenome which highlight the importance of using of human *in vitro* models for predicting effects in humans.

61. Prospective cohort studies in OBELIX are still underway. The first published study has indicated an inverse relationship between PCB 153 (a marker of PCB exposure) and birth weight in a meta-analysis of 12 European birth cohorts. Decreases in birth weight of 150 g with each 1 µg L^{-1} increase of PCB 153 in cord serum were observed. Interestingly, no association of 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE), the breakdown product of the organochlorine pesticide DDT, and birth weight was found in the meta-analysis, though at an individual cohort level, some cohorts did also observe a significant inverse relationship.

62. On-going work is currently examining the growth trajectories in children, and differentiating between pre- and postnatal exposure to EDCs. Breastfeeding is a major source of exposure in the developing infant that has largely been overlooked in epidemiological studies studying chemical-obesity links. A novel toxicokinetic model has been developed to simulate levels of persistent organic pollutants (POP) in children's blood that accurately estimates POP levels at 6 months and later based on maternal or cord blood

levels. Preliminary analyses using this model indicated divergent effects of pre- and postnatal exposure to POPs on growth trajectories in children.

63. The author identified a large number of other questions such as exposure to the complex mixtures, influence of chemicals on other putative mechanisms of obesity (such as circadian rhythms, inflammation or composition of gut microbiota) and the combined effect of chemical exposure and an adverse fetal environment (under or over nutrition)

Evidence for Obesogens: Interpretations and Next Steps by Gohlke and Allison (2013)

64. This editorial tried to reconcile the three presented perspectives on environmental chemicals as obesogens. Schug and Heindel suggested there was compelling evidence demonstrating many industrial and agricultural chemicals act as obesogens and provide hypotheses on how endocrine disruption, particularly during early development, could lead to obesity later in life. On the other hand, Sharpe and Drake were skeptical of the current evidence linking environmental chemicals to obesity, emphasizing two primary criticisms: (1) Animal model studies had largely relied on high dose levels that do not reflect routes and magnitudes of exposures experienced by humans and (2) Epidemiological studies linking chemicals to obesity were likely confounded by high fat diets, since fat in the diet was the primary route of exposure for many of the lipophilic environmental chemicals considered obesogens, as well as total food intake which will increase obesity. Unwilling to draw conclusions on the current evidence, Legler stressed the need for further research and introduced the OBELIX ("OBesogenic Endocrine disrupting chemicals: LInking prenatal eXposure to the development of obesity later in life") project, which is examining endocrine disrupters in the diet through a prospective birth cohort in four European countries and long-term animal studies using exposures that are comparable to those experienced in human populations.

65. These perspectives highlight the range of informed opinions even when the very same evidence is presented. For example, Schug/Heindel and Sharpe/Drake cited Tremblay *et al*. (2004), as evidence supporting and refuting organochlorines (OCs) as obesogens, respectively. Tremblay *et al*. measured sleeping metabolic rate (SMR) before and after a weight-reducing program and found that the decrease in SMR, beyond what would be predicted by weight loss alone—i.e., the adaptive reduction in thermogenesis upon weight loss, was positively correlated with OC plasma levels. Contrary to Schug and Heindel's interpretation of these data that OCs can lead to a higher probability of regaining lost pounds after weight loss, heightened adaptive thermogenesis predicts resistance to losing weight due to reduced energy expenditure. Alternatively, Sharpe and Drake state Tremblay's work suggests OCs increase thermogenesis, thus leading to increased weight loss, but in fact adaptive thermogenesis here refers to decreases in energy expenditure upon decreased energy intake. Yet another, possibly more straightforward interpretation of Tremblay's results would be that adaptive

thermogenesis is primarily determined by fat loss, and since ingested OCs are primarily stored in fat cells, greater concentrations of OCs are seen in blood after greater fat loss. Exposure and elimination of lipophilic compounds were determined by fat intake and fat loss, respectively, therefore epidemiological studies going forward should explicitly address potential confounding with both dietary fat intake and fat loss via weight reduction programs or other mechanisms, such as breastfeeding. Birth cohort studies may be informative in this regard, as long as sufficient variation in exposures can be captured across all levels of potentially confounding variables. Interestingly, initial OBELIX birth cohort results show an inverse relationship between PCB levels and birth weight. The next challenge is development of ethical and practical study designs to adequately control for potential confounding.

66. Despite the divergent interpretations of individual studies and the differences in the overall conclusions drawn in these three perspectives, it is clear we have imperfect knowledge. As Sharpe and Drake pointed out, the large number of positive association studies for BPA and obesity as evidence for causality belies the fact that plausibility is tenuous due to confounding by diet. Even longitudinal studies will have difficulty teasing apart the contribution of diet versus BPA exposure. Thus an important question is what type of evidence is critical to address the issue. Whilst a randomized controlled trial (RCT) would be optimal for determining causality, ethical concerns make RCTs unrealistic when only hypothesized adverse health effects exist with neither demonstrated nor even plausible benefits. Additionally, there are practical limitations, including the importance time of exposure during development, the long lag between exposure and effect and the suggestion of non-monotonic dose-response curves. Thus RCTs would not be feasible for evaluating environmental contaminants as obesogens.

67. Natural or quasi experimental study designs may offer alternative methods for satisfying some causality criteria when RCTs are not a reasonable option. For example, studies on hypothesized effects of breastfeeding and other factors on obesity highlight that these designs can offer inferential strength which is between that of RCTs and an observational epidemiological design.

68. The authors note the importance of making a clear distinction between drawing a conclusion on causality and use of evidence in making a policy decision. Current evidence may not be sufficient for drawing a conclusion on the obesogenic potential for some environmental contaminants. A decision to limit exposures, either individually or at the societal level, is a different question, which hinges on balancing the immediacy with which the decision may be necessary with the estimated benefits and risks associated with the decision.

Role of PPAR

69. A 2011 review by Janesick and Blumberg (Annex 4) sets out arguments for a hypothesised role of peroxisome proliferator-activated receptor gamma (PPARγ) as a key regulator of adipogenesis which is linked

to obesity and type II diabetes. This short review focused on recent evidence linking endocrine disrupting chemicals to PPARγ and examined the possible molecular mechanisms by which they may cause effects. The authors suggested that as PPARγ was a master regulator of adipogenesis, a logical hypothesis was that inappropriate activation of the receptor contributes to obesity. The authors suggested that an open question is whether most or all chemicals that activate PPARγ will ultimately be shown to be obesogenic.

70. Activation of the PPARγ receptor by certain pharmaceutical drugs, such as the TZDs, or xenobiotic compounds has been shown to stimulate adipogenesis *in vitro* and *in vivo*. Obesogens were chemicals that ultimately increase obesity through a variety of potential mechanisms, such as increasing the number of fat cells, up-regulating fat storage into existing fat cells, changing the amount of calories burned at rest, shifting energy balance to favor storage of calories or altering the mechanisms through which the body regulates appetite and satiety. The hypothesis was that these effects result from inappropriate activation of PPARγ. The authors considered that tributyltin was the first obesogen for which a definitive mechanism of action has been elucidated through its ability as a PPARγ and RXR activator. The authors noted that not all chemicals that activate PPARγ were adipogenic or correlated with obesity in humans. Citing the example of selective PPAR modulators, they noted that using activity on PPARγ was not a reliable surrogate for predicting adipogenic potential since some compounds were either receptor-selective or cell-selective (agonists in some cell types but not others due to differential recruitment of co-regulators). Since increased adipogenesis inevitably results in a concomitant increase in PPARγ expression, which may reflect the outcome, rather than the mechanism of obesogen action.

71. There were multiple mechanisms through which obesogens could interact with PPARγ without direct activation of the receptor. Ligand-independent mechanisms could act through obesogen-mediated post-translational modification of PPARγ causing receptor de-repression or activation. PPARγ was active in multipotent stem cells committing to the adipocyte fate during fat cell development. By modifying chromatin structure early in development, obesogens could influence the promoter activity of PPARγ, or the ability of PPARγ to bind to its target genes, ultimately biasing the progenitor pool towards adipocyte lineage. Through directly or indirectly activating PPARγ, increasing the levels of PPARγ protein or enhancing its recruitment of promoters of key genes in the adipogenic pathway obesogens may affect adipogenesis and obesity.

72. Even when an obesogenic chemical was demonstrated to be a PPARγ activator, how the ligand acted to increase fat cell number and lipid storage in humans was not apparent. Nor was the extent to which PPARγ activation was involved or required for the obesogenic phenotype. An important gap was understanding how prenatal and early life exposure to obesogenic chemicals could program exposed individuals to gain weight and what, if any, role modulation of PPARγ expression or activity had in this process.

Questions asked of the committee.

73. Members are asked to consider specific questions on the reviews and papers cited and some general questions on the obesogen hypothesis and needs for future work.

NIH workshop.

i. In light of the fact that the primary focus and context for the specific exposures varied widely do members consider that it was appropriate in the NIH Workshop to review not just obesity but metabolic syndrome and type 2 diabetes as well since obesity is a major risk factor for these diseases. Do members wish to comment on possible confusion that could arise from lacking a clear end point.

ii. Do members consider that asking experts to suggest relevant assay targets that could be included in Tox21 in the future to better screen for perturbations of these biological processes presupposed a linkage and could have introduced a possible evaluation bias

iii. Do members wish to consider on the definition or lack thereof of studies considered relevant and the strategy outlined in paragraph 8 for extracting findings from these studies.

iv. Do members consider that the evidence for mechanisms by which arsenic could influence pancreatic β-cell function and insulin sensitivity speculative and too inconsistent to provide a mode of action

v. Is the suggestion that several sulfonylurea herbicides and imidazole fungicides were also identified by the ToxRefDB search as possibly associated with causing type 2 diabetes inconsistent with the statement they belong to the same general chemical class as agents used to manage or being investigated as potential therapeutic agents for this disease.

vi. Do members agree with the conclusions of the NIH workshop that the existing literature supported the plausibility of the “obesogen” hypothesis and linkages between type 2 diabetes and exposures to certain chemical classes

vii. Do members wish to comment on the research recommendations from the NIH workshop including the suggested most appropriate end points to evaluate in human, animal, and mechanistic studies of diabetes and obesity (Tables 2–4 of Annex 1).

Chem trust report.

viii. Do members wish to comment on the approach taken and the conclusions reached, members may wish to consider the evidence presented for and against the hypothesis is complete and evaluated appropriately.

Obesity papers.

ix. Members are invited to comment on the papers overall and particularly the observation that the same data can be cited in support of and against the hypothesis.

x. Do members wish to comment on the point made that there are significant data gaps which need to be addressed before reaching conclusions

PPAR

xi. Members are invited to comment on this proposed mechanism of action and whether the suggestion that these effects result from inappropriate activation of PPARγ is feasible and how this can be distinguished from appropriate activation of the receptor.

General questions.

xii. Members are asked to consider whether they should make a more detailed evaluation of the obesogens hypothesis at this time or whether this is premature especially prior to the completion of the OBELIX project. Would it be more appropriate for the Secretariat to continue monitoring the subject and update the Committee on developments in future horizon scanning activities.

xiii. Do members wish to comment on the postulated role of epigenetic mechanisms and to what extent does this warrant further evaluation.

xiv. Would it be a more suitable subject for a future COT workshop

xv. If members consider that further evaluation is required what aspects would they wish to see covered in detail and what priority should be given to the work.

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Secretariat

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