



**HAL**  
open science

## **Obesity III: Obesogen assays: Limitations, strengths, and new directions**

Christopher D Kassotis, Frederick S Vom Saal, Patrick J Babin, Dominique Lagadic-Gossmann, Helene Le Mentec, Bruce Blumberg, Nicole Mohajer, Antoine Legrand, Vesna Munic Kos, Corinne Martin-Chouly, et al.

► **To cite this version:**

Christopher D Kassotis, Frederick S Vom Saal, Patrick J Babin, Dominique Lagadic-Gossmann, Helene Le Mentec, et al.. Obesity III: Obesogen assays: Limitations, strengths, and new directions. *Biochemical Pharmacology*, 2022, 199, pp.115014. 10.1016/j.bcp.2022.115014 . hal-03669590

**HAL Id: hal-03669590**

**<https://hal.science/hal-03669590>**

Submitted on 23 Sep 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Obesity III: Obesogen Assays: Limitations, Strengths, and New Directions**

2 Christopher Kassotis<sup>1</sup>, Frederick S. Vom Saal<sup>2</sup>, Patrick J. Babin<sup>3</sup>, Dominique Lagadic-Gossman<sup>4</sup>, Helene  
3 Le Mentec<sup>4</sup>, Bruce Blumberg<sup>5</sup>, Nicole Mohajer<sup>5</sup>, Antoine Legrand<sup>4</sup>, Vesna Munic Kos<sup>6</sup>, Corinne Martin-  
4 Chouly<sup>4</sup>, Normand Podechard<sup>4</sup>, Sophie Langouët<sup>4</sup>, Charbel Touma<sup>4</sup>, Robert Barouki<sup>7</sup>, Min Ji Kim<sup>8</sup>,  
5 Karine Audouze<sup>9</sup>, Mahua Choudhury<sup>10</sup>, Nitya Shree<sup>10</sup>, Amita Bansal<sup>11</sup>, Sarah Howard<sup>12</sup>, and Jerrold J.  
6 Heindel<sup>12</sup>

7  
8 <sup>1</sup> Institute of Environmental Health Sciences and Department of Pharmacology, Wayne State University,  
9 Detroit, MI 48202

10 <sup>2</sup>Division of Biological Sciences, The University of Missouri, Columbia MO, 65211

11 <sup>3</sup> Department of Life and Health Sciences, University of Bordeaux, INSERM, Pessac France

12 <sup>4</sup>Univ Rennes, Inserm, EHESP, Irset (Research Institute for Environmental and Occupational Health) –  
13 UMR\_S 1085, 35 000 Rennes, France

14 <sup>5</sup>Department of Developmental and Cell Biology, The University of California, Irvine, Irvine CA 92697

15 <sup>6</sup>Department of Physiology and Pharmacology, Karolinska Institute, Solna, Sweden

16 <sup>7</sup>Department of Biochemistry, University of Paris, INSERM, Paris, France

17 <sup>8</sup>University of Sorbonne Paris Nord, Bobigny, INSERM U1124 (T3S), Paris FR

18 <sup>9</sup>University of Paris, T3S, INSERM U1124, Paris France

19 <sup>10</sup>Department of Pharmaceutical Sciences, Texas A & M University, College Station, TX 77843

20 <sup>11</sup>College of Health & Medicine, Australian National University, Canberra, ACT, 2611, Australia

21 <sup>12</sup>Healthy Environment and Endocrine Disruptor Strategies, Commonweal, Bolinas CA 92924

22

23

24 Short title: Overall perspective on obesogen assays

25 Corresponding Author:

26 Christopher D. Kassotis, PhD

27 Institute of Environmental Health Sciences and  
28 Department of Pharmacology, School of Medicine

29 Wayne State University

30 2111 Integrative Biosciences Center

31 6135 Woodward Avenue

32 Detroit, MI 48236

33 Phone: 313-577-0170

34 Email: christopher.kassotis@wayne.edu

35

36 Publication Support:

37 Christopher Kassotis, NIH, R00ES030405

38 Patrick J. Babin, European Union Horizon 2020 Research and Innovation Program, Oberon #825712

39 Dominique Lagadic-Gossman, European Union Horizon 2020 Research and Innovation Program, Oberon  
40 #825712

41 Sophie Langouët, European Union Horizon 2020 Research and Innovation Program, Oberon #825712

42 Antoine Legrand, European Union Horizon 2020 Research and Innovation Program, Oberon #825712

43 Hélène Le Mentec, European Union Horizon 2020 Research and Innovation Program, Oberon #825712

44 Frederick S. vom Saal, NIH, R02ES02139

45 Charbel Touma, European Union Horizon 2020 Research and Innovation Program, Oberon #825712  
46 Robert Barouki, European Union Horizon 2020 Research and Innovation Program, Oberon #825712  
47 Amita Bansal, Diabetes Australia #S5610040  
48 Bruce Blumberg, NIH, R01ES023316, R01ES031139  
49 Karine Audouze, European Union Horizon 2020, Research and Innovation Program, Oberon #825712  
50 Vesna Munic Kos, Swedish Research Council for Sustainable Development (FORMAS)#2019-00375  
51 Normand Podechard, European Union Horizon 2020 Research and Innovation Program, Oberon #825712

52  
53

54 Disclosure Summary: None of the authors have anything to declare regarding writing this review.

55

56 **Abstract**

57 There is increasing evidence of a role for environmental contaminants in disrupting metabolic health in  
58 both humans and animals. Despite a growing need for well-understood models for evaluating adipogenic  
59 and potential obesogenic contaminants, there has been a reliance on decades-old *in vitro* models that have  
60 not been appropriately managed by cell line providers. There has been a quick rise in available *in vitro*  
61 models in the last ten years, including commercial availability of human mesenchymal stem cell and  
62 preadipocyte models; these models require more comprehensive validations but demonstrate real promise  
63 in improved translation to human metabolic health. There is also progress in developing three-  
64 dimensional and co-culture techniques that allow for the interrogation of a more physiologically relevant  
65 state. While diverse rodent models exist for evaluating putative obesogenic and/or adipogenic chemicals  
66 in a physiologically relevant context, increasing capabilities have been identified for alternative model  
67 organisms such as *Drosophila*, *C. elegans*, zebrafish, and medaka in metabolic health testing. These  
68 models have several appreciable advantages, most notably the size, rapid development, large brood sizes,  
69 and ease of high-resolution lipid accumulation throughout the organisms. They are anticipated to expand  
70 the capabilities of metabolic health research, particularly when coupled with emerging obesogen  
71 evaluation techniques as described herein.

72

73 **Abbreviations**

74 United States (US); white adipose tissue (WAT); peroxisome proliferator-activated receptor gamma  
75 (PPAR $\gamma$ ); sterol-regulatory element-binding protein-1 (SREBP-1); liver X receptor alpha (LXR $\alpha$ );  
76 glucocorticoid receptor (GR); retinoid X receptor-alpha/beta (RXR $\alpha/\beta$ ); estrogen receptor alpha (ER $\alpha$ );  
77 human preadipocytes (HPAd); Simpson-Golabi-Behmel syndrome (SGBS); mesenchymal stem cells  
78 (MSCs); Human multipotent adipose-derived stem cells (hMADS); toxicant-associated fatty liver  
79 diseases (TAFLD); non-alcoholic fatty liver disease (NAFLD); dimethylsulfoxide (DMSO); primary  
80 human hepatocytes (PHH); cytochrome P450 (CYP); aryl hydrocarbon receptor (AhR); constitutive  
81 androstane receptor (CAR); pregnane X receptor (PXR); di(2-ethylhexyl) phthalate (DEHP);  
82 dichlorodiphenyltrichloroethane (DDT); bisphenol A (BPA); polychlorinated biphenyls (PCBs);  
83 tetrabromobisphenol A (TBBPA); days post-fertilization (dpf); endocrine disrupting chemicals (EDCs);  
84 insulin-producing cells (IPCs); brown adipose tissue (BAT); uncoupling protein 1 (UCP1); OLTAM  
85 (ODD-Luc based Thermogenic Activity Measurement); ODD (oxygen-dependent degradation); hypoxia-  
86 inducible factor 1 alpha (HIF1 $\alpha$ ); quantitative structure-activity relationship (QSAR); Toxicity Testing in  
87 the 21st Century (Tox21); Organization for Economic Cooperation and Development (OECD); New  
88 approach methodologies (NAMs); Adverse Outcome Pathways (AOPs); integrated approaches to testing  
89 and assessment (IATAs); National Institute for Environmental Health Sciences (NIEHS); PEPPER  
90 (Public-privatE Platform for the Pre-validation of Endocrine disRuptors).

91

92

93

94

95

96

97

98

99

100 **Essential Points**

101 There are increasing novel capabilities to identify and assess obesogens.

102

103 There is still a reliance on using well-defined models with unclear translation to human health.

104

105 There is still a need for comprehensive validations of novel metabolic health models.

106

107 Computational models show some promise in future predictions and assessments of obesogens.

108

109

110

## 111 1. Introduction

112

113 Over the last several decades, the global prevalence of metabolic disorders, specifically obesity, has risen  
114 at an alarming rate. Despite extensive investments in exploring interventions to address this health trend,  
115 the incidence rates continue to rise. In the United States (US), 8.9% of infants and toddlers [1, 2], 18.5%  
116 of 2-19 year old's [1, 2], and 42.4% of adults (20+) [3] are currently classified as obese, with an  
117 additional 31.2% of the adult population classified as overweight [4]. Obesity consumes >\$200 billion of  
118 the US health care expenditure annually and also drives increased risks of various comorbidities (e.g.,  
119 type II diabetes, cardiovascular disease, hypertension) [5-8]. High societal costs [8, 9] have driven support  
120 for research into causal factors, including exposure(s) to environmental contaminants. Previous research  
121 estimated extremely high economic costs of obesity, diabetes, and associated health costs reasonably  
122 attributable to environmental contaminants in the European Union [9], even when only considering five  
123 chemicals for which sufficient epidemiological data were available.

124

125 As detailed in the companion review, Obesity II, "obesogens" are environmental chemicals that increase  
126 the size of white adipose tissue (WAT) stores in the body as a result of exposure *in vivo* [10, 11].  
127 Chemicals that can induce adipogenesis in cellular models *in vitro* but have not yet been shown to  
128 increase WAT stores *in vivo* are designated as potential obesogens [12]. Considering the complexity of  
129 human chemical exposures, the increasing reports of obesogens, and the rising incidence of metabolic  
130 disorders, it is critical to identify and validate comprehensive models (*in silico*, *in vitro*, and *in vivo*) for  
131 the identification and evaluation of obesogens. One of the major challenges in the obesity field is to  
132 develop a robust set of tests that can reveal adipogenic and/or obesogenic properties of chemicals and  
133 have strong predictive capacity in humans. These tests should be in line with the 3R principles (i.e.,  
134 reducing the number of animals, refining experiments to minimize the number of animals used, and  
135 replacing animal experiments where possible). Practically speaking, the high costs of animal experiments  
136 limit the use of mammals in screening for potential obesogens. This supports an urgent need for increased

137 use of lower-order (*in silico*, *in vitro*) testing to prioritize higher-order (*in vivo*) testing. There is also an  
138 urgent need for new *in vivo* models that are less time and cost-intensive to support *in vivo* testing that is  
139 still required for the tens of thousands of chemicals used in commerce. While the number and diversity of  
140 cellular models of adipocyte differentiation and metabolic health is increasing, these require  
141 comprehensive validation to determine the strengths and weaknesses of each for their relevance to human  
142 metabolic health

143  
144 Despite the potential limitations of available animal models to reproduce human disease fully, they help  
145 evaluate exposure pathways, generation of *in vivo* metabolites, elucidating tissue and/or disease biology,  
146 and underlying molecular mechanisms involved in adverse health outcomes. The choice of the animal  
147 model should consider the degree to which the outcomes being examined are relevant to humans and the  
148 sensitivity of these outcomes to environmental chemicals. The relevance of the model to human health  
149 depends considerably on the evolutionary conservation of biological processes impacted by candidate  
150 chemical or pharmacological molecules between humans and the animal model used. It is likely that a  
151 single test might not reveal all relevant properties and that a battery of tests should be developed. This set  
152 of tests should address the following issues: 1) evaluate *in vivo* obesity according to its different  
153 characteristics, including the type and importance of different adipose depots; 2) reveal *in vitro* and *in*  
154 *silico* assays/models that reliably predict obesity; 3) identify *in vivo* biomarkers that are predictive of  
155 obesity, and 4) account for delays between exposure(s) to putative obesogens and the appearance of a  
156 phenotype.

157  
158 Mammalian models have been relied on for metabolic health testing due to clear translation of adipose  
159 physiology. However, non-mammalian model species are increasingly appropriate for the screening and  
160 rapid identification of chemicals and mixtures and the exploration of disease mechanisms. Knowledge  
161 acquired from non-mammalian model systems (e.g., vertebrates such as teleost fish and invertebrates such  
162 as flies and worms) can provide insights into mechanisms involved in regulating lipid metabolism and

163 transport processes that have been intractable by other approaches [13]. Due to the conservation of lipid  
164 metabolism processes among vertebrates, the zebrafish model has become an attractive alternative to  
165 rodents, with lower costs and time investments.

166

## 167 **2. In vitro assays**

168 The most well-established lower-order testing protocols are the adipogenesis cell assays, although newly  
169 developed cell models have allowed an increasing breadth of metabolic disruption assessment (Figure 1).  
170 Several *in vitro* models were developed in various species (primarily human and murine) to identify  
171 potential obesogens [14, 15]. These models generally assess three endpoints: commitment to the  
172 adipocyte lineage (via multipotent MSC models), preadipocyte proliferation (proliferation of early-stage  
173 adipocyte lineage cells), and differentiation into mature adipocytes (adipogenesis; generally determined  
174 via quantification of intracellular triglyceride accumulation).

175

### 176 *2.1 Preadipocyte models*

177 Preadipocytes are already committed to the adipocyte lineage and thus can be used to examine both  
178 proliferation (*via* nuclear staining) and adipogenesis (*via* triglyceride quantification). These cells are in an  
179 early stage of adipocyte development and require activation of signaling pathways to promote further  
180 development/maturation. Adipogenesis can be achieved by treating cells with a “differentiation cocktail”  
181 that contains a variety of hormonal and/or growth factors to initiate the process. These factors are often  
182 different between laboratories, but generally always include a mixture of fetal bovine serum, insulin, and  
183 isobutylmethylxanthine (IBMX); some laboratories also include thyroid hormone and/or glucocorticoids,  
184 though the presence of these and concentrations varies widely. Once the cocktail is removed, the relative  
185 roles of various test chemicals in the role of differentiation (assessed *via* triglyceride accumulation) and  
186 proliferation (of adipocyte precursor cells) can be assessed [16-19].

187

188 The 3T3-L1 mouse cell line was isolated and described in the 1970s and has been utilized for decades as



189 an *in vitro* screen to examine the mechanisms regulating adipogenesis and evaluate potential adipogenic  
190 chemicals [16, 17, 20]. This cell line has been used to carefully explore mechanisms promoting and  
191 underlying various stages of adipogenesis [21, 22] and has been shown to appropriately select chemicals  
192 for further testing (linking *in vitro* results to *in vivo* health outcomes; e.g., bisphenol A and tributyltin)  
193 [23-31]. While this line has been well-characterized [21], its sourcing can be unreliable [32, 33]. For  
194 example, nuclear receptor expression related to adipogenesis is markedly different between lots and  
195 sources of this cell line [32]. These and other cell line integrity issues can contribute to discrepancies in  
196 replication efforts between laboratories [34, 35]. We recently undertook an interlaboratory reproducibility  
197 effort of 3T3-L1 responses to a positive control chemical (rosiglitazone) and three blinded test chemicals  
198 [35]. While the determination of “active” *versus* “inactive” were consistent across the ten participating  
199 laboratories, the potencies and efficacies of the blinded chemical responses varied by orders of  
200 magnitude. The cross-over study design allowed for determinations of the sources of variation, and our  
201 results demonstrated that inconsistencies of the cell line sources and differentiation protocol differences  
202 promoted most of the variation. Thus the harmonization of protocols across laboratories may help support  
203 consistent reporting of adipogenic results [35]. Despite these limitations, 3T3-L1 cells remain the most  
204 popular model for assessing adipogenic outcomes. Specifically, numerous publications have assessed  
205 bisphenols [26, 32, 36], brominated and organophosphate flame retardants [37-39], per and  
206 polyfluoroalkyl substances [40, 41], and diverse other environmental contaminants [20, 24, 37] and  
207 mixtures [42] using this cell model. There is an emerging interest in determinations of whether  
208 environmental contaminant exposures promote the development of normal or abnormal adipocytes, and  
209 some preliminary data has begun to evaluate this. For example, BPA enhanced levels of leptin,  
210 interleukin-6, and interferon gamma in mature adipocytes, resulting in hypertrophic adipocytes with  
211 impaired insulin signaling, increased pro-inflammatory cytokine production, and reduced glucose  
212 utilization [43].

213

214 The OP9 mouse bone marrow-derived stromal cell line is another established preadipocyte model [19, 44]

215 that allows faster differentiation (2-3 *versus* 10-14 days). This cell line is considered to be a later stage  
216 preadipocyte than 3T3-L1 cells because it expressed key adipogenic factors such as CCAAT/enhancer-  
217 binding proteins alpha and beta, peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ), sterol-  
218 regulatory element-binding protein-1 (SREBP-1), perilipin, and other adipocyte markers that are not  
219 expressed in basal 3T3-L1 cells before adipogenic induction [19]. Therefore, OP9 cells can be induced to  
220 accumulate triglycerides within two days, differentiation is not diminished by maintenance in culture at  
221 high cell density, their adipogenic potential is maintained for >100 passages, and they do not require  
222 contact inhibition and reversion to clonal expansion before initiating the differentiation induction [19].  
223 These characteristics suggest a promising model with lower time and cost investments, though this does  
224 require careful validation to understand the translation of responses to human health effects. We have  
225 reported that these cells do differentially express nuclear receptors relative to 3T3-L1 cells, including  
226 PPAR $\gamma/\alpha$ , liver X receptor alpha (LXR $\alpha$ ), glucocorticoid receptor (GR), retinoid X receptor-alpha/beta  
227 (RXR $\alpha/\beta$ ), and estrogen receptor alpha (ER $\alpha$ ) [32]. As a result, responsiveness to adipogenic chemicals in  
228 OP9 cells is significantly different from 3T3-L1 cells, characterized by lower responsiveness *via*  
229 activation of GR and greater responsiveness *via* the RXR pathway [32, 45]. While still an uncommon  
230 model for assessing obesogens, OP9 cells have been used to evaluate bisphenols [32], pesticides [45], and  
231 other environmental contaminants [45].

232  
233 More recently, several human preadipocyte models have become available that hold promise for future  
234 evaluations of adipogenicity by environmental contaminants. Since the basis for much of our  
235 understanding of adipogenesis has been evaluated using the murine 3T3-L1 cells, utilizing these newer  
236 human models may help elucidate any species-specific differences that may be present. Many companies  
237 now supply primary human preadipocytes (HPAd) isolated from several human subcutaneous depots,  
238 visceral depots, and/or adipose surrounding the heart. Moreover, suppliers also provide source-specific  
239 HPAd cells, i.e., those sourced from donors with normal, overweight, or obese body mass indices and  
240 those with or without diabetes (e.g., see, <https://www.zen->

241 bio.com/products/cells/subcutaneous\_adipocytes.php). These discrete preadipocyte populations allow  
242 more targeted questions and potentially a better molecular understanding of adipogenesis. However,  
243 human preadipocyte cell models are cryopreserved at the end of primary culture. They can generally be  
244 propagated at most two additional passages before losing their ability to differentiate into mature  
245 adipocytes [46, 47]. As such, these models, while potentially more translationally relevant to human  
246 health, are extremely costly, as numerous cryopreserved vials are needed to complete any well-designed  
247 experiment (e.g., multiple biological replicates). Limitations aside, researchers have begun to utilize  
248 human preadipocytes to assess adipogenic and anti-adipogenic effects of botanical and biological  
249 mixtures [48-50], bisphenols [51], and flame retardants [38].

250  
251 The Simpson-Golabi-Behmel syndrome (SGBS) cell line addresses some of these limitations of using  
252 primary human preadipocytes. These cells were isolated from an infant with an extremely rare (250  
253 reported cases) metabolic health condition characterized by excess growth; this infant demonstrated  
254 expanded subcutaneous fat depots, and a sample of this tissue was obtained postmortem [52]. Profiling  
255 these cells suggests that they can be maintained and retain robust differentiation capability over 50  
256 passages [53], a significant advantage over normal human donor preadipocytes, and profiling has  
257 suggested morphological, biochemical, and functional similarities to differentiated adipocytes from  
258 healthy subjects [52, 54]. These cells also transiently express brown adipocyte markers [55-57],  
259 suggesting that this cell line might be useful for assessments of adipocyte browning. Proteomic and  
260 transcriptomic analyses of SGBS cells have been used to evaluate the molecular underpinnings of SGBS  
261 differentiation, with >1100 proteins and >300 genes differentially expressed in differentiated cells relative  
262 to undifferentiated [58]. However, some research comparing this model to existing models has suggested  
263 notable differences. Metabolomics and lipidomics profiling revealed a diverse grouping of lipid classes  
264 markedly changed throughout the differentiation process, suggesting a radically different metabolite  
265 profile than previously observed in 3T3-L1 cells [59]. SGBS cells have been used to evaluate the  
266 adipogenic effects of various bisphenols [60], though have not yet seen frequent use in this context. Other

267 human cell lines obtained from tumors or transformed can be differentiated into either white (Lisa, LS-14,  
268 AML-1, Chub-S7) or brown (PAZ6) adipocytes [61], but their use in toxicology is rare [60].

269

## 270 *2.2 Mesenchymal stem cells (MSCs)*

271 Another option in assessing adipogenesis is the utilization of multipotent mesenchymal stromal stem cells  
272 (mesenchymal stem cells, MSCs). MSCs are multipotent cells that can assess adipocyte lineage  
273 commitment in addition to adipocyte differentiation [18, 62, 63]. MSCs are isolated from either bone  
274 marrow or adipose tissue, and cells from both sources have been used to assess adipogenesis. The use of  
275 MSC models has been reviewed previously in the context of obesogens and their potential impacts on cell  
276 commitment and subsequent differentiation [64]. Recent work described a novel protocol for separately  
277 evaluating adipogenic commitment and subsequent differentiation in primary MSCs [63], previously  
278 described for the C3H10T<sub>1/2</sub> murine stem cell model [65, 66]. This protocol allows a complete  
279 characterization of potential obesogens and their role in disrupting cell commitment and differentiation.  
280 While the focus has been on evaluating effects on the adipocyte lineage, a growing body of research has  
281 begun to evaluate potential chemical impacts on osteogenic development using these models [67-70].  
282 Some limited research has evaluated chemical impacts on development down the chondrogenic,  
283 myogenic, or other cell lineages [64]. Human MSCs are readily available from diverse vendors, although  
284 murine models are also routinely used [45, 70-72].

285

286 Recent research elegantly described protocols for distinguishing assays to evaluate adipogenic lineage  
287 commitment and subsequent adipocyte differentiation [63]; briefly, cells can be pre-treated with test  
288 chemicals prior to the differentiation cocktail exposure. These pre-treated cells can be subsequently  
289 exposed to the differentiation cocktail and evaluated at the end of the differentiation window. The extent  
290 of triglyceride accumulation can be compared with standard adipogenesis plates; chemicals with effects  
291 on commitment should have equivalent effects to those differentiated for the full two weeks, whereas  
292 cells without effects on commitment should not accumulate more triglycerides than the vehicle control in

293 the commitment assays, regardless of effects in the standard adipogenesis assay [63].  
294  
295 The human MSCs lack the issues inherent in the primary human preadipocyte models; they can be  
296 maintained in culture for several more passages, have less variability in sourcing, and are easier to isolate  
297 and culture, increasing the utility of this model. This should lead to an increased reliance on human MSCs  
298 for adipogenic *in vitro* testing. However, rigorous reproducibility assessments and comprehensive  
299 validation testing are still needed to ensure accurate translation to and/or prediction of *in vivo* and human  
300 health outcomes. Diverse bisphenols [72-74] and their mixtures [75], flame retardants [18], parabens [76],  
301 and other environmental contaminants [63, 77-79] have been evaluated using MSC models. Research in  
302 female MSCs demonstrated that RXR agonists attenuated glucose uptake; blunted adiponectin expression;  
303 promoted a sustained interferon signaling, inhibiting markers of adipocyte browning; and unlike  
304 activation of PPAR $\gamma$ , failed to downregulate proinflammatory and profibrotic transcripts [77]. As the  
305 authors described, these data implicated RXR agonists in the development of dysfunctional white adipose  
306 tissue that could potentially exacerbate obesity and/or diabetes risk *in vivo*. Future research is needed to  
307 evaluate these functional differences in adipocyte physiology to determine more subtle effects of  
308 obesogenic contaminants. There has also been some initial research to evaluate the interplay between  
309 lineage commitment, suggesting that exposures to certain chemicals can not only commit cells to the  
310 adipocyte lineage but can also suppress the osteogenic lineage [45]; this interplay between different cell  
311 lineages is an area of research that still requires further investigation and mechanistic assessment.  
312  
313 Human multipotent adipose-derived stem cells (hMADS), obtained from human infant adipose tissue,  
314 have also been used to study the effects of aryl hydrocarbon receptor ligands that demonstrated an  
315 inflammatory response in pre- and adipocytes, a phenomenon observed in obesity [80]. hMADS were also  
316 used to screen 49 contaminants prioritized through ToxCast screening, reporting 26 active chemicals  
317 across diverse chemical groups (i.e., pesticides, phenolics, phthalates, etc.) [81].  
318

### 319 2.3 Spheroid adipocyte models

320 Spheroid cell cultures of both MSCs and preadipocytes are being developed and evaluated [82-87]. These  
321 culture techniques may allow some inherent benefits over the traditional adherent monolayer cultures.  
322 Spheroid culture of adipocyte models may improve differentiation efficiency relative to monolayer  
323 cultures [82-86, 88], reducing time and cost investment. The fundamental goal of spheroid models is to  
324 maintain greater *in vivo* or whole tissue-relevant signaling than monolayer models. Indeed, several papers  
325 have demonstrated greater adipogenic and osteogenic gene expression relative to monolayer cultures and  
326 a down-regulation of stemness markers [82, 83]. Other researchers have demonstrated increased plasticity  
327 of spheroid constructs through multiple generations of these cells able to commit to and differentiate into  
328 numerous cell lineages [89]. This plasticity might signal a greater variance in these models that requires  
329 further investigation. While these models have received no apparent use for the interrogation of putative  
330 obesogens, they have been demonstrated to exhibit improved relevance to the *in vivo* condition [90].  
331 Specifically, researchers have demonstrated that human unilocular vascularized adipocyte spheroids have  
332 unilocular morphology and large lipid droplets, and these cells develop key features of adipocyte  
333 dysfunction (e.g., insulin resistance, impaired lipolysis, and disrupted adipokine secretion; [90, 91]) and  
334 respond to stress (toxin or culture-related) by secreting pro-inflammatory adipokines [92]. These spheroid  
335 cultures also maintain expression of markers specific to certain adipocyte types (e.g., brown) for longer  
336 than is possible in 2D culture [92]. These 3D cultures also exhibit more physiologically relevant gene  
337 expression (>4500 differentially expressed genes relative to 2D culture) and lipid profiles of >1000 lipid  
338 species resemble the *in vivo* condition [93]. As such, these models may allow for a clearer understanding  
339 of adipose physiology than was possible with monolayer cultures and hence requires further evaluation  
340 and comprehensive validation and testing; this should also include evaluation of known adipogenic and/or  
341 obesogenic contaminants to compare responses with existing models.

342

### 343 2.4 Liver cell assays

344 Obesogens are also known to target liver (either directly or indirectly) and promote metabolic diseases  
345 such as toxicant-associated fatty liver diseases (TAFLD) or non-alcoholic fatty liver disease (NAFLD);  
346 thus, there is a need to have accurate *in vitro* hepatocyte models for testing chemicals. Liver cell assays  
347 are frequently used as surrogate models to predict *in vivo* hepatotoxicity related to chemicals and decipher  
348 the determinants of NAFLD development and progression. The use of various hepatocyte models for  
349 evaluating NAFLD and other metabolic disorders has been covered recently in detail [94-97]. These  
350 models have been used to evaluate diverse environmental contaminants, including bisphenols [98, 99],  
351 phthalates [99-101], pesticides [102], other environmental contaminants [99, 101], and therapeutics [103]  
352 for effects on NAFLD and other metabolic dysfunction.

353  
354 Among many liver cell lines, HepG2 cells a human hepatoma cell line commonly used for drug  
355 metabolism and hepatotoxicity studies. HepG2 cells express certain differentiated hepatic functions like  
356 lipoprotein metabolism, triglyceride metabolism, bile acid synthesis, glycogen synthesis, or insulin  
357 signaling, making them a useful tool for some studies targeting hepatotoxicity and drug metabolism  
358 [104]. HepG2 cells exposed to a low concentration of BPA alter lipid metabolism, mitochondrial function  
359 and promote lipid accumulation leading later one to steatosis [105]. Co-incubation of HepG2 with fatty  
360 acids palmitic acid and oleic acid, induced lipid accumulation in a dose-dependent manner which will  
361 contribute to steatosis [106].

362  
363 Comparatively, human THLE-2 and murine AML12 cell lines are derived from healthy liver cells and  
364 express characteristics of normal adult liver epithelial cells [107]. Insulin receptor expression was low in  
365 THLE-2 cells relative to AML12 and HepG2 cells, suggesting disparities in their application to insulin  
366 receptor signaling. Gluconeogenesis and hepatokine expression was impaired in both THLE-2 and  
367 AML12 cells; while expression of Angiopoietin Like 4 (ANGPTL4) was regulated by PPAR $\delta$  activation  
368 similarly across THLE-2, AML12, and HepG2 cells, only HepG2 cells reflected the *in vivo* environment

369 with regulation by cAMP [107]. These models have been utilized to evaluate fatty acid induced lipid  
370 droplet accumulation and the presence and causes of heterogeneity in the lipid droplet content [108],  
371  
372 The most prevalent human liver cell line is HepaRG. HepaRG cells can differentiate into hepatocyte-like  
373 and biliary-like phenotypes after dimethylsulfoxide (DMSO) (1.75 - 2%) exposure, and possess the ability  
374 to stably express several liver-specific genes such as albumin, aldolase B, CYP2E1 and CYP3A4 [109].  
375 Changes in metabolites related to energy metabolism, oxidative stress, and insulin resistance have also  
376 been observed in differentiated HepaRG cells supplemented with an oleate/palmitate mixture [110].  
377 These are consistent with alterations observed in the liver tissues of human patients and animal models of  
378 NAFLD [111, 112]. Altogether, these data further support the suitability of the fatty acid-supplemented  
379 HepaRG model to study the impact of obesogens on steatosis progression towards steatohepatitis in the  
380 context of the “two-hit” model [113]. In line with these data, an oleate/stearate mixture is sufficient to  
381 decrease the expression of CYP1A1, 1A2, 1B1 and decrease their activity after steatosis induction [114].  
382 These results corroborate data obtained from NAFLD rodent models, especially regarding CYP1A1 and  
383 1A2 [115-117].  
384  
385 In addition, several 3D liver culture models have also been developed to create a cell environment closer  
386 to *in vivo* conditions. In 3D cell cultures, cell growth and interaction with surrounding conditions exhibit  
387 higher differentiation and benefit from more extended culture than 2D cultures [118]. When cultured as  
388 3D spheroids, HepaRG cells express genes involved in lipoprotein metabolism, energetic lipid synthesis,  
389 gluconeogenesis, glycolysis, and bile acid metabolism, liver-specific functions, and xenobiotic  
390 metabolism enzymes [119, 120].  
391  
392 Primary human hepatocytes (PHH) are increasingly used to predict drug metabolism and liver enzyme  
393 induction in humans. However, PHH have inherent limitations: scarce and unpredictable availability,  
394 limited growth activity and lifespan, and early and variable phenotypic alterations in 2D culture.



395 Moreover, liver-specific functions, particularly cytochrome P450 (CYP) activities and their  
396 responsiveness to prototypical inducers, are not maintained with increasing time of culture. Liver-specific  
397 functions also usually decrease with time in culture and are differently altered [121, 122]. Cultivated in a  
398 3D collagen matrix, they proliferate, form hollow spheroids, and undergo robust hepatic differentiation.  
399 They can be maintained in this state for at least 28 days without decreasing survival rate and cellular  
400 polarity and require fewer cells to generate spheroids than 2D cultures [123]. PHH 3D-spheroid models  
401 co-cultured with liver sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, increase human  
402 hepatocyte functionality (increased mRNA expression of APOB, CYP3A4, and albumin). Essential  
403 factors such as spheroid size, time in culture, and culture media composition affect basal levels of  
404 xenobiotic metabolism and liver enzyme inducibility *via* activators of hepatic receptors such as the aryl  
405 hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), and pregnane X receptor (PXR)  
406 [124]. Various co-culture techniques have also been developed for liver cell assays to recreate more tissue  
407 or disease-relevant environments for the evaluation of disease biology and toxicology [125].

408  
409 Similarly, primary murine hepatocytes (PMHs) are readily isolated through rapid protocols and thus have  
410 improved availability relative to PHH [126]. PMHs have been well-described as a model to assess fat  
411 deposition, inflammatory responses, and mechanistic interrogation of fatty acid induced lipid  
412 accumulation by diverse contaminants [127-129].

### 413 414 *2.5 Muscle cell assays*

415 While skeletal muscle is the main tissue responsible for utilization of glucose and is the main site of the  
416 development of insulin resistance, the impact of toxicants on skeletal muscle has not been extensively  
417 studied. Detecting effects *in vitro* can be difficult due to the specific cell culture requirements and  
418 stimulation of skeletal muscle fibers required to mimic physiological function. Since skeletal muscle  
419 plays a critical role in developing metabolic diseases, any chronic disturbances in muscle cells may  
420 contribute to insulin resistance and subsequent obesity.

421  
422 The most widely used *in vitro* myocyte model is the murine myoblast cell line, C2C12. These cells can be  
423 differentiated into myotubes (immature muscle cells) over several days. BPA and estradiol have been  
424 demonstrated to suppress myogenic differentiation by inhibiting Akt signaling in C2C12 cells [130],  
425 potentially disrupting ER signaling. Tolyfluanid alters insulin signaling, mitochondrial function, and  
426 protein synthesis in C2C12 cells in a manner dependent on fatty acid levels [131]. The rat myoblast cell  
427 line, L6, has a longer differentiation time relative to C2C12 cells, as well as appreciable differences in  
428 mitochondrial respiration and glucose utilization [132]. In L6 rat myotubes, di(2-ethylhexyl) phthalate  
429 (DEHP) exposure was shown to affect insulin receptor expression, GLUT4 expression, as well as glucose  
430 uptake and oxidation, indicating that it may negatively influence insulin signaling [133]. The pesticides  
431 dichlorodiphenyltrichloroethane (DDT) and lindane impair insulin signaling in L6 myotubes, promoting  
432 insulin resistance-like conditions [134].

433  
434 Human and rodent primary myoblasts are also used. However, they are unsuitable for extended cultures  
435 and more extensive screening studies due to relatively low numbers of cells obtained at a relatively high  
436 cost. Some polychlorinated biphenyls (PCBs) have been shown to inhibit myogenic differentiation of  
437 primary murine myoblasts and L6 cells [135]. In primary murine myoblasts differentiated to myotubes,  
438 low micromolar concentrations of BPA and tetrabromobisphenol A (TBBPA) were shown to affect  
439 calcium signaling and resting potential. In a similar study, using rabbit skeletal muscle microsomes, BPA  
440 and TBBPA were shown to differently affect the function of proteins involved in calcium signaling [136].

441  
442 Notably, there are distinct differences between mature muscle tissue and myotubes derived from myoblast  
443 cell lines or primary myoblasts [132]. Myotubes have lower energy demand, lower oxidative  
444 phosphorylation, higher glycolysis, and lower insulin responsiveness [137]. There is a considerable  
445 knowledge gap regarding the effects of environmental chemicals in more complex and physiologically  
446 relevant skeletal muscle systems, which require additional validations.

447

### 448 3. *In vivo* assays

449 While *in vitro* mechanistic studies are a critical component in environmental chemical research, these  
450 studies cannot replace the need for *in vivo* integrative models, particularly for adverse health outcomes  
451 that develop later in life following developmental exposures. Research examining the environmental  
452 health consequences of exposure to environmental chemicals using animal models has demonstrated that  
453 some adverse health effects of chemical exposures reported in humans are also apparent across other  
454 vertebrates [138]. These findings are essential for understanding the impact of environmental chemicals,  
455 including obesogens, across all vertebrates [139]. These tests are critical because the classification of  
456 obesogens into different classes according to the strength of evidence is highly dependent on the tests  
457 used.

458

459 Beyond the classical rodent *in vivo* models used to investigate human obesity, new models have emerged  
460 based on alternative model organisms, *e.g.*, bony fishes, worms, and flies [140] (Figure 2). These model  
461 organisms, including *Danio rerio* (zebrafish), *Caenorhabditis elegans* (*C. elegans*; roundworm), and  
462 *Drosophila melanogaster* (fruit flies), offer several advantages to accurate discernment of the metabolic  
463 processes involved in metabolic diseases such as obesity [141]. These organisms share small size, large  
464 numbers of progeny, relatively rapid development, and sequenced genomes. They are well suited to  
465 moderate throughput screening of chemicals to study metabolic diseases [142-146]. Moreover, most  
466 genes and gene families implicated in metabolic diseases are conserved among flies, worms, zebrafish  
467 and humans [144]. Below we present a short overview of the utility of each model and some summarized  
468 obesogenic chemical evaluation using these emerging models (Table 1).

469

#### 470 3.1 *Danio rerio* (Zebrafish)

471 Zebrafish, a small tropical freshwater fish native to South Asia (*e.g.*, India and Bangladesh), has found  
472 wide use in almost all areas of biological research [147, 148]. Zebrafish is one of the most widely used

473 models to study metabolic dysfunction. They have indeed all the critical organs that regulate energy  
474 homeostasis and metabolism, including adipose tissue, digestive organs, i.e., pancreas and liver, and  
475 skeletal muscles, all physiologically and anatomically like humans [141, 149, 150]. The rapid  
476 development of zebrafish promotes metabolically functional organs only a few days post-fertilization  
477 (dpf; e.g., pancreas and liver develop around three dpf). Organogenesis and biological processes can be  
478 easily monitored due to the extra-uterine development and the semitransparency of the embryo and larva  
479 stages that persist until a relatively late stage of development [151].

480  
481 Zebrafish store excess neutral triglycerides in lipid droplets within white adipocytes similar to mammals  
482 [152] and have well-described anatomically, physiologically, and molecularly distinct adipose depots  
483 throughout their bodies [153-155]. This contrasts with *Drosophila* and *C. elegans*, where fat is stored in  
484 non-specialized cells (within the fat body or within the intestine, respectively) that carry out several other  
485 functions besides lipid storage [156]. Regulations of body weight, appetite, lipid, and sugar homeostasis  
486 share similar mechanisms between humans and zebrafish and are similarly affected by endocrine  
487 disrupting chemicals (EDCs) [145, 157, 158]. The development of WAT starts in the pancreatic and  
488 abdominal adipose depots, then in various cranial and ocular depots, and finally expands throughout the  
489 fish. The appearance correlates with the size rather than the age of the fish [159-161]. The first adipocytes  
490 develop from 8-12 dpf or at a minimal larval size of approximately 5 mm [160].

491  
492 Zebrafish obesity models enable the evaluation of diet, chemical or genetic, phenotypic modifiers through  
493 several different techniques [162-165]. Measurement of total body triglycerides may be used as an  
494 indicator for evaluating adiposity and/or obesity progression [161]. Adipocytes can also be visualized and  
495 quantified by lipid staining with the Oil Red O neutral dye or with various fluorescent lipophilic dyes  
496 (e.g., Nile Red, Lipid Green) in live fish, adult zebrafish sections, or fixed zebrafish larvae. Since  
497 zebrafish larvae are transparent, live-imaging and fluorescent staining allow ready detection and  
498 quantification of intracellular lipid droplets and adipose tissue, including its regional body distribution

499 [166, 167]. These methodological advantages have been exploited for developing a bioassay to evaluate  
500 the obesogenic properties of chemicals in zebrafish larvae [161]. Zebrafish models can also help assess  
501 specific windows of sensitivity during life as well as transgenerational effects of obesogens [168-170] and  
502 can be used to study the interaction between the diet composition and metabolic health effects promoted  
503 by subsequent chemical exposures [114, 152, 159, 160, 171]. Interesting recent research demonstrated  
504 that long-term dietary vitamin D deficiency promoted stunted growth and increased central adiposity via  
505 both adipocyte hypertrophy and hyperplasia in both visceral and subcutaneous depots [172]. Through  
506 lipidomics analysis, these fish were demonstrated to have increased bioactive lipids that seemed to be  
507 mediated through disrupted endocannabinoid signaling [173].

508  
509 Zebrafish have been widely applied to obesogenic chemical testing, with expanding capacity for  
510 modulation of diverse metabolic disrupting effects [27, 169, 174-177]. Among other obesogenic chemical  
511 evaluations, developmental exposure of bisphenol S in combination with overfeeding promoted increased  
512 triacylglycerol and visceral adiposity via disrupted lipid metabolism [175], while BPA exposures both  
513 transiently and persistently disrupted food intake, increased body weights, and disrupted gene expression  
514 related to glucose and lipid metabolism [165]. Halogenated BPA analogs also promoted lipid  
515 accumulation in zebrafish larvae in a manner correlated with their activity as zebrafish PPAR $\gamma$  agonists  
516 [27]. Developmental exposures to nonylphenol and nonylphenol polyethoxylates increased body weights  
517 and adiposity (in both viscera and subcutaneous adipose depots) and disrupted energy expenditure [79].  
518 Tributyltin exposure has been described to increase body weights, hepatic triglycerides, and  
519 hepatosomatic index, along with disrupting genes related to adipogenesis, lipogenesis, and diverse other  
520 metabolism and growth-related pathways [178] as well as increasing adiposity [161]. Developmental  
521 cadmium exposures have also been demonstrated to increase lipid accumulation, though this effect was  
522 transient (observed at one and two months post fertilization but was no longer observed by 3.5 months  
523 [177]). Perfluorooctane sulfonate (PFOS) exposures have also been described to increase adiposity and

524 disrupt pancreatic islet morphology and area in developmentally exposed zebrafish, along with increasing  
525 fatty acid concentrations and disrupting PPAR gene expression [169].

526

### 527 *3.2 Oryzias latipes (Medaka)*

528 The Japanese rice fish, also known as the medaka, are a valuable model for environmental chemical and  
529 epigenetic transgenerational research [179]. Similar to zebrafish, this model can be used for estimating  
530 adipose tissue volumes and the effects of nutritional factors (dietary soy sauce oil) or various  
531 environmental chemicals such as per/polyfluoroalkyl substances and tributyltin chloride [180-182].  
532 However, they lack the thorough characterization of adipose depots and the transparent bodies that  
533 zebrafish benefit from. They have also been utilized for determining transgenerational effects on  
534 metabolic health outcomes such as lipid metabolism [183]. Research using medaka has also evaluated  
535 chemical exposures and effects on bone formation [184], suggesting a potential strength for this model in  
536 the evaluation of differential MSC lineage commitment.

537

538 Medaka have not yet been widely used in obesogenic chemical evaluations, but some preliminary research  
539 suggests utility in this model for diverse obesogenic endpoints. Specifically, exposure of medaka to both  
540 tributyltin and perfluorooctane sulfonate (PFOS) individually promoted adipose accumulation in larvae,  
541 with mixtures of these two obesogens resulting in enhanced effects (even below the individual no-effect  
542 concentrations) [181]. In related research, tributyltin exposures disrupted signaling pathways related to  
543 PPAR signaling, hormonal metabolism, and genes related to obesity in humans via mRNA-Seq analysis  
544 in exposed zebrafish [185]. Similarly, BPA exposure was reported to disrupt genes related to lipid  
545 metabolism (cholesterol and lipid synthesis, regulation, and transport, etc.) in a sex-specific manner [186].

546

### 547 *3.3 C. elegans (Roundworm)*

548 The roundworm is a small nematode living in temperate soil environments that has been used as a model  
549 organism since the 1960s in everything from developmental biology to neurodegenerative disease and

550 aging. Although *C. elegans* is generally considered genetically and physiologically distant from humans,  
551 several studies have shown that the main regulatory pathways of energy homeostasis are shared between  
552 mammals and nematodes [144, 187, 188]. These advantages make *C. elegans* a suitable *in vivo* model to  
553 identify compounds that modulate fat storage and promote obesity [141, 189]. Both simple fluorescence  
554 (Nile red or Sudan-black probes) and biochemical (triglyceride assays) techniques can be used to quantify  
555 lipid amount and fat storage in these worms [188]. In addition, genetic approaches using mutant or  
556 transgenic animals can help evaluate molecular mechanisms underlying metabolic health effects [187,  
557 188]. Moreover, *C. elegans* can be readily used to measure food intake and energy expenditure [188,  
558 190]; several diets, food-derived or nutraceutical compounds, and fat-increasing compounds have been  
559 described to modulate fat accumulation [189-191]. Limitations of this model include lower conservation  
560 of biological pathways with humans and a lack of particular organs and circulatory systems [192]. *C.*  
561 *elegans* also lack PPAR $\gamma$ , though they do express orthologs of both PPAR $\alpha$  and  $\delta$ , and have no  
562 identifiable homolog for leptin [193, 194]. Perhaps unsurprisingly, they thus have no cells specifically  
563 designed for lipid storage (i.e. adipocytes), though they do still store lipids, primarily in intestinal and  
564 epidermal skin-like cells, which are comprised of diverse saturated, monounsaturated, and polyunsaturated  
565 fatty acids [193]. This model has also been used to assess transgenerational effects, with research  
566 demonstrating that starvation of the parental generation promoted disrupted metabolism in the F3  
567 generation, whereas BPA exposures resulted in transgenerational modulation of epigenetic germline  
568 silencing through up to five subsequent (non-exposed) generations (reviewed in [195]).  
569  
570 Despite these limitations, this model has been utilized widely in better understanding the genetics of fat  
571 accumulation, storage, and obesity [194, 196], and has been applied to obesogenic chemical evaluation  
572 successfully. Specifically, methylmercury exposure promotes triglyceride accumulation, lipid storage, and  
573 alter feeding behaviors [197], erythromycin promotes increased fat content and triacylglycerol levels as  
574 well as promoting overeating, presumably mediated through stimulation of serotonin, dopamine, and  
575 acetylcholine and/or disruption of lipogenesis and lipolysis [198]. Recent research demonstrated a non-

576 monotonic increase in overall fat deposition and triglyceride content following bisphenol S exposures,  
577 along with modulation of fat synthesis and fatty acid oxidation gene expression [199].

578

### 579 *3.4 Drosophila melanogaster (Fruit fly)*

580 The fruit fly is one of the most used model organisms throughout biological research. The small size,  
581 short generation time, low cost, ease of breeding, and a large panel of genetic tools have spurred use in  
582 genetic and developmental biology research [192, 200]. Many studies have demonstrated the usefulness  
583 of this model in nutrition and obesity studies based on the manipulation of diet composition and genes  
584 involved in nutrient sensing and regulation of energy balance [201]. Although this model is anatomically  
585 different from mammals, many organ systems perform similar functions relative to mammals. For  
586 example, the fruit fly fat body covers metabolic functions of liver and adipose tissue (e.g., fat and  
587 carbohydrate storage). Instead of a fully differentiated pancreas, there are neurosecretory insulin-  
588 producing cells (IPCs), which allow carbohydrate and lipid homeostasis *via* the production and secretion  
589 of an insulin-like peptide [146, 201]. Few studies have utilized this model to evaluate potential obesogens  
590 and/or obesity biology, though its suitability for evaluating endocrine impact(s) on development and  
591 fertility is well accepted [202]. The efficiency of this model in assessing obesogenic properties of EDCs is  
592 highlighted by several studies demonstrating alterations of lipid homeostasis with chemical exposure  
593 (e.g., DEHP) and subsequent increase in lipid/adipose accumulation and/or transgenerational effects [203-  
594 205].

595

### 596 *3.5 Rodents*

597 A critical issue in selecting an animal model is whether the outcomes examined are relevant to human  
598 anatomy, physiology, molecular mechanisms and show homology with humans, which has historically  
599 driven a reliance on rodent models (e.g., rats and mice). The use of rodents in metabolic health research is  
600 well-described and assessed by several previous reviews [206-208]. Here we will address other  
601 considerations for *in vivo* model organism research revealed through comprehensive evaluations in rodent



602 models. Many of these factors have yet to be evaluated or considered for the emerging models described  
603 above but will need to be assessed as they are increasingly used.

604  
605 Dozens of publications have clearly delineated the use of the rodent model in metabolic health research.  
606 A number of studies (reviewed in [207, 208]) have explicitly described the use of hypercaloric and/or  
607 high fat diets to promote metabolic disorders and the clear translation of this preclinical model to human  
608 metabolic syndrome. However, other approaches, such as creating a crowded uterus in pregnant mice due  
609 to prior hemiovariectomy, have also been used to generate metabolically abnormal intrauterine growth  
610 restricted (IUGR) and macrosomic offspring in the same litter [209].

611  
612 There are diverse genetic models of obesity, including *db/db* mice (leptin receptor mutation that promotes  
613 higher body weights, triglycerides, and cholesterol, hyperinsulinemia, and impaired glucose tolerance),  
614 *ob/ob* mice (leptin gene mutation resulting in inactive leptin protein and promoting obesity,  
615 hyperinsulinaemia and hyperglycaemia), *fa/fa* diabetic fatty rats (different leptin receptor mutation  
616 promoting hyperinsulinaemia, hypertriglyceridaemia, and increased serum inflammatory markers), and  
617 Otsuka Long-Evans Tokushima fatty rats (Pancreatic acini cells insensitive to cholecystokinin, which  
618 controls food intake, promoting obesity, hypertriglyceridaemia, impaired glucose tolerance), that have  
619 been described in detail previously [206]. Rodents can be robust models for body weight, adiposity,  
620 development of specific adipose depots, measurement of diverse lipid classes, glucose and insulin  
621 signaling, inflammatory markers, blood pressure, controlled measurement of food and water intake and  
622 metabolic activity, as well as NASH and NAFLD, among other metabolic outcomes [206].

623  
624 *3.6 Use of inbred vs. outbred models*

625 Genetic diversity of model organisms (inbred *versus* outbred) can be an essential design consideration for  
626 chemical contaminant studies. Researchers may select an inbred rodent strain without background genetic  
627 variation to study the epigenetic basis of phenotypic diversity (e.g., inheritance of an epigenetic trait)

628 [210]. In contrast, a researcher may choose an outbred rodent (e.g., CD-1) for the genetically diverse  
629 background to assess toxicant-induced effects more rigorously. However, there are concerns that  
630 laboratory outbred rodent strains differ substantially between vendors and relative to bona fide outbred  
631 animals. Inbred rodents do not represent the spectrum of sensitivity required to model genetically diverse  
632 human populations accurately. For example, males at puberty have considerable heterogeneity in rodent  
633 responsiveness to estrogens [211]. The C57BL/6J inbred strain is exquisitely sensitive to estradiol after  
634 puberty relative to other mouse strains/stocks and exhibits hyper-estrogenization during fetal life, which  
635 becomes apparent in behavioral assays [212]. Interestingly, C57 mice are insensitive to xenoestrogens  
636 administered *via* the dam compared to the outbred, hyper-fertile CD-1 mouse, which exhibits high  
637 sensitivity fetal-neonatal response to xenoestrogens [213]. Given this, the choice of strain used can have  
638 demonstrable impacts on endpoint measurements.

639

### 640 *3.7 Animal feed as a source of variability*

641 Animal feed can be a substantial source of variability in toxins, phytoestrogens, sources of fats, and other  
642 components. Open formula feeds provide the proportion of nutrients, which is intended to reduce, but not  
643 eliminate, batch-to-batch variability. Closed formula (constant nutrition) feeds just provide information  
644 about the amount of protein, fat and fiber, but the sources may vary due to price and availability [214,  
645 215]. Thus, the choice of feed used in animal studies, impacted by price, can be a critical source of  
646 variability in outcomes of health-related research and can also be the basis for studies that do not replicate  
647 prior results [216]. For example, publications by Thigpen and colleagues reported that a batch of constant  
648 nutrition rodent feed (Purina® 5002) containing elevated levels of phytoestrogens (focusing on the soy  
649 isoflavones genistein and daidzein) interfered with the ability to see estrogenic effects of a positive  
650 control chemical, the potent estrogenic drug diethylstilbestrol (DES). However, DES effects were  
651 observed with another batch of 5002 feed that had much lower phytoestrogen levels. The rat strain used  
652 also mattered, with Sprague-Dawley rats showing no effect of use of soy feed, while the CD-1 mouse (the

653 model used by the National Toxicology Program), is, as discussed below, very sensitive to components of  
654 feed [217].

655  
656 This observation by Thigpen demonstrated that there can be significant batch-to-batch variability of  
657 phytoestrogen levels in laboratory animal feed with presumably the same nutrient profile; a constant level  
658 of soy protein in different batches of a feed can have markedly different levels of phytoestrogens, which  
659 vary in soy based on many environmental factors [216]. It has been assumed for some time that the only  
660 issue of concern with soy-based feeds was variability in the soy phytoestrogens genistein and daidzein,  
661 but findings described below suggest other components of soy-based feeds (e.g., contaminated fish meal,  
662 source of lipid) may also lead to significant differences in phenotype in mice. Second, the study revealed  
663 that specific batches of feed could promote replication failure relative to most prior studies reporting that  
664 DES (a known human carcinogen) disrupted development in mice, just as it did in humans [218].

665 Developmental exposure to DES also promoted obesity during later adulthood in mice maintained on a  
666 soy-based (NIH31) open formula feed [219]. This demonstrates that a core issue should be whether the  
667 feed used is resulting in an inability to see effects in response to treatments that others are reporting. Not  
668 surprising is that industry-funded research on BPA, which claimed to be a replication of findings from  
669 multiple laboratories [220], in fact, had used 5002 feed [221, 222]. This led to a failure to demonstrate a  
670 BPA-induced effect in both CF-1 mice and Crl:CD Sprague-Dawley (CD-SD) rats. This research also  
671 failed to demonstrate effects of DES with this food (included as positive control) [221], suggesting an  
672 inappropriate model to detect BPA-induced effects [223].

673  
674 In other studies, the expected developmental effects of DES were again shown not to occur in CD-1 mice  
675 fed 5002 feed, but were found if the mice were fed the constant nutrition, soy-based Purina<sup>®</sup> 5008/5001  
676 breeder and maintenance feeds, respectively. Specifically, relative to Purina<sup>®</sup> 5008 fed to pregnant CD-1  
677 mice, the 5002 feed significantly estrogenized and elevated fetal serum estradiol in fetuses. Critically, the  
678 5008 feed had >50% higher total estrogenic activity (detected in a human breast cancer cell bioassay) as

679 well as higher amounts of genistein and daidzein relative to the 5002 feed, substantiating that 5002 feed  
680 interfered with finding DES effects, but this was not mediated by elevated genistein and daidzein or total  
681 estrogenic activity as initially proposed [224].

682  
683 In addition to problems related to the use of soy-based 5002 feed, feeding casein-based low phytoestrogen  
684 5K96 feed to pregnant CD-1 mice also elevated endogenous serum estradiol in fetuses compared to CD-1  
685 mice fed Purina® 5008; 5K96 casein feed thus also promoted estrogenization of mouse fetuses, similar to  
686 effects in mice exposed as fetuses to xenoestrogens such as DES or BPA [225]. Relevant to this review,  
687 the 5K96 feed resulted in morbid obesity in adult CD-1 male mice (all internal organs were encased in  
688 fat) compared to Purina 5008/5001 or Harlan Teklad 8604, another soy-based constant nutrition feed  
689 [225, 226].

690  
691 Another example of feed-based impact on a supposed “non-replication” experiment was when prior  
692 metabolic effects of BPA and DES were not found is a study in which the control CD-1 mice were  
693 morbidly obese and did not show the previously reported effects of fetal exposure to BPA or DES [227]  
694 while maintained on the casein-based AIN93G feed [228]. The fetal mice whose mothers were fed casein-  
695 based 5K96 or soy-based 5002 feeds potentially had elevated aromatase (estrogen synthetase) activity,  
696 thus elevating fetal estradiol levels, compared to other soy-based feeds. Various flavonoids and lignans  
697 have been reported to inhibit aromatase activity in a human preadipocyte cell culture assay [229],  
698 although the components of the different feeds that caused these effects remain unknown.

699  
700 There have been many articles published about the issue of non-replication in laboratory research, mostly  
701 attempting to sensationalize the problem [230], but clearly, there are issues, such as variability in feed,  
702 that are a major contributing factor in non-replication in laboratory animal research. The above findings  
703 demonstrate the critical importance of, whenever possible, including a positive control in toxicological or  
704 pharmacological studies that will provide information about the sensitivity and validity of the assays and

705 results [223]. The vast diversity of animal feed components, including the casein or soy backbone and  
706 multiple sources of protein and lipids, can markedly impact research findings related to metabolic health.

707

### 708 *3.8 The role of positive controls in animal model selection*

709 A National Toxicology Program (NTP) panel addressed animal models for EDCs or drug research. It  
710 stated: “Because of clear species and strain differences in sensitivity, animal model selection should be  
711 based on responsiveness to active endocrine agents of concern (i.e., responsive to positive controls), not  
712 on convenience and familiarity.” The rat strain (CRL: CD(SD)) is used by many investigators to examine  
713 gestational exposure to estrogenic chemicals and drugs, although this rat strain required over a 15-fold  
714 higher dose of ethinylestradiol to show a response relative to women [231]. It is well known that selecting  
715 for very high fecundity (CD-SD rats average 14-15 pups per litter), results in low sensitivity to estrogenic  
716 drugs and chemicals [232].

717

718 It is also possible that the characteristics selected for in the generation of the CD-SD rat strain, with large  
719 litter size and accelerated postnatal growth, may make them resistant to contaminant exposures, reducing  
720 their future sensitivity and usefulness as a model; this strain is generally used in all FDA and in many  
721 commercial laboratory toxicology studies. Some strains have undergone selection for large litter sizes for  
722 over 100 generations in commercial laboratories, with the largest 5-10% of litters selected every  
723 generation for >100 generations, regardless of whether they were exposed to pesticides (in feed or used in  
724 the colony), xenoestrogens in their cage materials, or diseases in the colony, etc. The result is laboratory  
725 animal strains that are precocious, excellent breeders and produce large litters. However, the laboratory  
726 animal suppliers selected large litter animals not sensitive to environmental chemicals [211, 232]. Thus,  
727 before proceeding with experiments using environmental chemicals such as potential obesogens, it is  
728 critical to examine the sensitivity of the animal model to appropriate positive controls (e.g., DES for  
729 estrogenic testing) for the endpoint examined to ensure that each experimental design is sensitive to the

730 environmental chemical being examined.

731

### 732 *3.9 Animal housing*

733 The caging used in an experiment is an additional key factor. This was clearly described in studies of  
734 BPA, the monomer used to make polycarbonate cages and bottles. Due to harsh washing of the cages,  
735 BPA was found to leach from the polycarbonate cages; this was further shown to expose both control and  
736 intervention animals to this xenoestrogen, negatively influencing the experimental determinations of  
737 successful meiosis in mouse oocytes [233-235]. It is also worth noting that the vast majority of aquatic  
738 housing systems use polycarbonate; there is likely to be leaching of BPA from these and potential  
739 recirculation of the chemical throughout the system. While some alternatives do exist [e.g., polysulfone  
740 (PS) or glass], they are often cost-prohibitive. Polycarbonate (PC) consists of BPA molecules linked by  
741 ester bonds that are subject to hydrolysis under elevated temperature or either high or low pH. PS is a co-  
742 polymer of BPA and bisphenol S (BPS) that is linked by ether bonds and is stable under temperature and  
743 pH conditions that hydrolyze BPA bonds in polycarbonate, though PS cages are more expensive. It is  
744 essential to ascertain the potential impacts of the housing materials (for rodents, also water bottles) on  
745 testing estrogenic or other metabolism disrupting chemicals.

746

### 747 *3.10 Assays for detecting thermogenic brown fat activity*

748 Beige and brown thermogenic fat produces heat during non-shivering thermogenesis to regulate body  
749 temperature by burning calories (i.e., glucose and lipids) [236]. These tissues help regulate glucose and  
750 lipid levels, making them high-priority targets for future therapeutics in the treatment and prevention of  
751 obesity and other metabolically related diseases [237]. The functionality of beige and brown fat and the  
752 discovery that these tissues exist in adults have made the development of reliable assays a critical step to  
753 better quantify and harness their therapeutic potential as well as to identify chemicals that promote or  
754 inhibit function.

755

756 The energy expenditure in beige and brown adipose tissue (BAT) is made possible through the activity of  
757 uncoupling protein 1 (UCP1) in brown and beige fat, which uncouples mitochondrial respiration from  
758 ATP production, leading to the generation of heat [237]. Reporter systems that focus on UCP1 levels  
759 have been developed to measure the activity of thermogenic fat and have been used as a screening tool to  
760 identify novel small molecules that can induce thermogenesis within these tissues. Specifically, the  
761 ThermoMouse model measures thermogenesis via luciferase activity linked to levels of UCP1 expression  
762 in BAT following environmental stimuli (e.g., decreased temperatures) [238], which has also been  
763 adapted as an *in vitro* assay to screen small molecules for luciferase activity [238]. This assay has  
764 supported screening of potential drug targets that promote UCP1, and which could provide a foundation  
765 for future BAT-mediated drug therapies that could induce thermogenesis and energy expenditure [239-  
766 243].

767  
768 The OLTAM (ODD-Luc based Thermogenic Activity Measurement) system was developed to assay the  
769 activity of UCP1 independent thermogenesis in beige and BAT. In this *in vivo* model, a transgenic mouse  
770 that expressed the ODD (oxygen-dependent degradation) domain of hypoxia-inducible factor 1 alpha  
771 (HIF1 $\alpha$ ), tagged with luciferase, was used to measure hypoxia. Hypoxia has been shown to take place  
772 during nonshivering thermogenesis in beige and brown fat and is an indicator of thermogenesis [244]. An  
773 *in vitro* system was developed using the stromal vascular fraction of isolated brown adipocytes from these  
774 mice to measure cell-based thermogenic activity [244]. These cells could be used to evaluate the action of  
775 chemicals on the function of thermogenic beige and brown adipocytes.

776  
777 Measuring changes in heat generated within BAT offers another tool to assay thermogenic activity.

778 Noninvasive imaging techniques lack sensitivity and specificity due to the distance between the  
779 instrument and the tissue, and invasive techniques lack sensitivity due to their inability to directly and  
780 safely insert into BAT and their inability to detect more minute temperature fluctuations [245]. Xenon-  
781 enhanced computed tomography enabled accurate measurement of BAT within mice due to the lipophilic

782 preference of xenon gas [246], which has been further enhanced through later research [245].  
783 ERthermAC, a small molecule fluorescent dye that responds to changes in intracellular heat, is another  
784 tool that has been found to assay chemically stimulated thermogenesis in both rodent and human brown  
785 adipocytes [247], and has provided evidence comparable to existing indirect methods of measurement.  
786  
787 Lastly, UCP1-expressing brown adipose cells isolated from supraclavicular depots in humans have  
788 revealed that the molecular makeup of these cells more closely resembled mouse beige adipocytes than  
789 brown adipocytes [248]. In addition, humans who initially possessed no BAT, were found to create new  
790 BAT within the supraclavicular region. This suggests that human BAT is derived from the browning of  
791 beige fat. One could develop assays based on these cells to identify chemicals that promote or inhibit the  
792 production of these thermogenic adipocytes.

793

#### 794 4. *In silico* tests

795 Computational strategies offer promising tools for developing animal-free models for human risk  
796 assessment of obesogens. Traditional computational methods using structural information of chemicals  
797 (quantitative structure-activity relationship (QSAR), Read Across) have already been outlined as a  
798 general strategy for non-animal testing approaches, for example, by the US National Research Council  
799 (Tox21, Toxicity Testing in the 21st Century) [249] and the Organization for Economic Cooperation and  
800 Development (OECD) guidelines. New approach methodologies (NAMs), including *silico* methods, are  
801 increasingly important in toxicant risk assessment [250].

802

803 With the recent advance in omics and high throughput screening, the amount of information on  
804 gene/protein activity in response to obesogenic chemicals has expanded substantially, thereby enabling  
805 the development of innovative approaches such as integrative systems biology/toxicology models.  
806 Systems toxicology uses advanced bioinformatics and statistical tools to integrate heterogeneous data  
807 types (functional genomic profile of obesogens, protein-protein interactions, protein-tissue associations,



808 disease annotations, etc.) to mimic the complexity of the biological organization, to identify  
809 uncharacterized putative associations between an obesogen and its biological targets, and therefore to  
810 prioritize further experimental testing, thereby associating these chemicals with the disease [251, 252].

811  
812 Adverse Outcome Pathways (AOPs) are structured frameworks representing relationships between  
813 molecular initiating events, key events, and adverse outcomes. The OECD proposed AOPs to enable  
814 robust mechanistic evidence for chemical safety and risk assessment [253]. However, for chemical risk  
815 assessments, a pragmatic approach has been proposed for applying AOP criteria in evaluating the safety  
816 of a chemical [254], since a comprehensive understanding of the initiating events and molecular pathways  
817 linking chemicals to adverse outcomes is unrealistic; for a chemical such as BPA with over 10,000  
818 publications and clearly understood to result in adverse effects [255], understanding all of the AOPs is  
819 still a work in progress. AOPs describe and connect data from various sources, i.e., databases and the  
820 scientific literature. Key information used to build AOPs can also be gathered using computational  
821 approaches based on artificial intelligence, such as frequent itemset mining and text mining [256]. AOP-  
822 helpFinder is a recent hybrid tool that combines text mining and graph theory, helping identify the  
823 existing linkages between variables (e.g., an obesogen and a biological event) by automatically screening  
824 the available scientific abstracts [257]. Using this tool, it was possible to link exposure to bisphenol S  
825 with obesity [258]. Integrative systems toxicology modeling and text mining can also link obesogens to  
826 AOPs, as proposed recently for bisphenol F [259].

827

## 828 **5. The Future of Screening for Obesogens**

829 A single approach or assay will not yield all the information needed to identify and classify obesogens.  
830 Data from epidemiological studies should be integrated with experimental data from animal models to  
831 support the evidence for the obesogenic potential of an identified chemical. It is advisable to adopt a  
832 tiered approach to identify and characterize EDCs, which can ultimately inform their classification as  
833 obesogens, which has been proposed previously [260]. For example, if robust biomarkers such as

834 epigenetic modifications (e.g., DNA methylation), growth factors, or metabolites are identified through *in*  
835 *vivo* experimental studies, they can be matched with findings from human studies. *In vitro* methods that  
836 assess these changes will support prioritized screening for putative obesogens, which can then be  
837 classified accordingly. Structured frameworks, such as the integrated approaches to testing and  
838 assessment (IATAs), allow categorization of different tests that support the linkage of a chemical with an  
839 adverse outcome and with the different events leading to that outcome. IATAs are expected to be used for  
840 large scale obesogen testing and appear to be more time- and cost-effective than current approaches [261].  
841 Additional *in vitro* tests are needed, including assays that will develop and characterize brown and beige  
842 adipocytes to be used to define further the sites and actions of potential and actual obesogens.

843  
844 Approaches like this have been previously attempted using the ToxCast dataset. The National Institute for  
845 Environmental Health Sciences (NIEHS) hosted a workshop in 2011 to develop models for predicting  
846 obesogenic and/or diabetogenic outcomes using ToxCast and Tox21 data [262]. Expert panels developed  
847 (among others) a model to predict chemicals likely to promote adipocyte differentiation. An early  
848 application of this model reported poor performance in predicting both active and inactive adipogenic  
849 chemicals and suggested that better validation of primary high throughput screening assays was required  
850 before using ToxCast data for this purpose [62]. Later analysis updated the predictive model and reported  
851 more promising effects [81]. Computational modeling cannot substitute for experimental (*in vitro* and *in*  
852 *vivo* studies) but can help prioritize obesogens, assess human health risks and trigger new epidemiological  
853 and experimental studies. To be useful for screening purposes, computational models need to be grounded  
854 in real-world data and continually refined such that predicted activities match the results of *in vitro* and *in*  
855 *vivo* screening assays.

856  
857 Indeed, there is consensus regarding the need for standardized testing methods to identify new chemicals  
858 that trigger metabolic dysfunction. In this context, initiatives like the French PEPPER (Public-privatE  
859 Platform for the Pre-validation of Endocrine disRuptors characterization methods, <https://ed-pepper.eu>)

860 platform may facilitate development of pre-validated methods and assays in toxicology for identification  
861 of novel EDCs [263]. In Europe, a collaborative group of eight projects, named EURION [264], was  
862 established in 2019. EURION aimed to develop integrative tests to identify new EDCs. Among  
863 EURION's projects, three projects focus on obesity and metabolic disorders (OBERON [265], GOLIATH  
864 [266], and EDCMET [267]), which are expected to deliver standardized batteries of tests for the  
865 identification of novel obesogens.

866  
867 As the field of obesity and adiposity research develops, more research will likely utilize some of the  
868 alternative models described above. While historically less utilized than rodents, these models have some  
869 advantages that are likely to see increased use in the coming years. Among these are the relatively lower  
870 cost and rapid development of assays and models that may allow for superior chemical mixture  
871 assessments than using rodent models. *In vitro* models have also continued to expand, with an anticipated  
872 shift to greater use of normal human cell models, three-dimensional culture techniques, and co-cultures  
873 techniques that may recreate the physiology present in the tissue microenvironment more accurately.  
874 Recent advances in high content analysis provide promising grounds for increased throughput of  
875 adipogenesis models, which would enable the screening of larger number of chemicals and their mixtures  
876 with increased sensitivity and the possibility to differentiate the changes in adipocyte number as well as  
877 size [42, 75]. Predictive models are still early in development but have shown some promise in predicting  
878 likely active adipogenic and/or obesogenic chemicals. Predictive models based on key concepts for  
879 obesogens (such as those recently described for EDCs and hepatotoxicants [268, 269]) are likely to  
880 support determinations of obesogens and their causal mechanisms of action. They should be prioritized on  
881 an international level, such as the OECD.

882

883

884 **Acknowledgements:**

885

886 Graphical abstract produced using Biorender.com.

887  
888

## References

- 889 [1] C.M. Hales, M.D. Carroll, C.D. Fryar, C.L. Ogden, Prevalence of Obesity Among Adults and Youth:  
890 United States, 2015-2016, National Center for Health Statistics, Hyattsville, MD, 2017.
- 891 [2] A.C. Skinner, S.N. Ravanbakht, J.A. Skelton, E.M. Perrin, S.C. Armstrong, Prevalence of Obesity and  
892 Severe Obesity in US Children, 1999-2016, *Pediatrics* (2018).
- 893 [3] Hales CM, Carroll MD, Fryar CD, O. CL, Prevalence of obesity and severe obesity among adults:  
894 United States, 2017–2018, NCHS Data Brief, no 360, National Center for Health Statistics, Hyattsville,  
895 MD, 2020.
- 896 [4] C.D. Fryar, M.D. Carroll, J. Afful, Prevalence of Overweight, Obesity, and Severe Obesity Among  
897 Adults Aged 20 and Over: United States, 1960-1962 Through 2017-2018, in: N.H. E-Stats (Ed.) 2020.
- 898 [5] R.A. Hammond, R. Levine, The economic impact of obesity in the United States, *Diabetes Metab*  
899 *Syndr Obes* 3 (2010) 285-95.
- 900 [6] D. Hamilton, A. Dee, I.J. Perry, The lifetime costs of overweight and obesity in childhood and  
901 adolescence: a systematic review, *Obes Rev* 19(4) (2018) 452-463.
- 902 [7] A. Goettler, A. Grosse, D. Sonntag, Productivity loss due to overweight and obesity: a systematic  
903 review of indirect costs *BMJ Open* 7 (2017) e014632.
- 904 [8] A. Biener, J. Cawley, C. Meyerhoefer, The Impact of Obesity on Medical Care Costs and Labor  
905 Market Outcomes in the US, *Clin Chem* 64(1) (2018) 108-117.
- 906 [9] J. Legler, T. Fletcher, E. Govarts, M. Porta, B. Blumberg, J.J. Heindel, L. Trasande, Obesity,  
907 Diabetes, and Associated Costs of Exposure to Endocrine-Disrupting Chemicals in the European Union,  
908 *The Journal of clinical endocrinology and metabolism* 100(4) (2015) 1278-88.
- 909 [10] A.S. Janesick, B. Blumberg, Obesogens: an emerging threat to public health, *American journal of*  
910 *obstetrics and gynecology* 214(5) (2016) 559-65.
- 911 [11] J.J. Heindel, F.S. vom Saal, B. Blumberg, P. Bovolin, G. Calamandrei, G. Ceresini, B.A. Cohn, E.  
912 Fabbri, L. Gioiosa, C. Kassotis, J. Legler, M. La Merrill, L. Rizzir, R. Machtinger, A. Mantovani, M.A.  
913 Mendez, L. Montanini, L. Molteni, S.C. Nagel, S. Parmigiani, G. Panzica, S. Paterlini, V. Pomatto, J.  
914 Ruzzin, G. Sartor, T.T. Schug, M.E. Street, A. Suvorov, R. Volpi, R.T. Zoeller, P. Palanza, Parma  
915 consensus statement on metabolic disruptors, *Environmental health : a global access science source* 14  
916 (2015) 54.
- 917 [12] J.J. Heindel, B. Blumberg, M. Cave, R. Machtinger, A. Mantovani, M.A. Mendez, A. Nadal, P.  
918 Palanza, G. Panzica, R.M. Sargis, L.N. Vandenberg, F.S. Vom Saal, *Metabolism disrupting chemicals*  
919 *and metabolic disorders*, *Reprod Toxicol* 68 (2017) 3-33.
- 920 [13] P.J. Babin, G.F. Gibbons, The evolution of plasma cholesterol: direct utility or a "spandrel" of  
921 hepatic lipid metabolism?, *Prog Lipid Res* 48(2) (2009) 73-91.
- 922 [14] S.P. Poulos, M.V. Dodson, G.J. Hausman, Cell line models for differentiation: preadipocytes and  
923 adipocytes, *Exp Biol Med* (Maywood) 235(10) (2010) 1185-93.
- 924 [15] F.J. Ruiz-Ojeda, A.I. Ruperez, C. Gomez-Llorente, A. Gil, C.M. Aguilera, Cell Models and Their  
925 Application for Studying Adipogenic Differentiation in Relation to Obesity: A Review, *Int J Mol Sci*  
926 17(7) (2016).
- 927 [16] H. Green, O. Kehinde, An established preadipose cell line and its differentiation in culture. II.  
928 Factors affecting the adipose conversion, *Cell* 5(1) (1975) 19-27.
- 929 [17] H. Green, M. Meuth, An established pre-adipose cell line and its differentiation in culture, *Cell* 3(2)  
930 (1974) 127-33.
- 931 [18] H.K. Pillai, M. Fang, D. Beglov, D. Kozakov, S. Vajda, H.M. Stapleton, T.F. Webster, J.J.  
932 Schlezinger, Ligand binding and activation of PPARgamma by Firemaster(R) 550: effects on  
933 adipogenesis and osteogenesis in vitro, *Environmental health perspectives* 122(11) (2014) 1225-32.
- 934 [19] N.E. Wolins, B.K. Quaynor, J.R. Skinner, A. Tzekov, C. Park, K. Choi, P.E. Bickel, OP9 mouse  
935 stromal cells rapidly differentiate into adipocytes: characterization of a useful new model of adipogenesis,  
936 *J Lipid Res* 47(2) (2006) 450-60.

937 [20] R.M. Sargis, D.N. Johnson, R.A. Choudhury, M.J. Brady, Environmental endocrine disruptors  
938 promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation, *Obesity (Silver*  
939 *Spring)* 18(7) (2010) 1283-8.

940 [21] X. Guo, K. Liao, Analysis of gene expression profile during 3T3-L1 preadipocyte differentiation,  
941 *Gene* 251(1) (2000) 45-53.

942 [22] M. Fu, T. Sun, A.L. Bookout, M. Downes, R.T. Yu, R.M. Evans, D.J. Mangelsdorf, A Nuclear  
943 Receptor Atlas: 3T3-L1 adipogenesis, *Mol Endocrinol* 19(10) (2005) 2437-50.

944 [23] H. Masuno, T. Kidani, K. Sekiya, K. Sakayama, T. Shiosaka, T. Yamamoto, K. Honda, Bisphenol A  
945 in combination with insulin can accelerate the conversion of 3T3-L1 fibroblasts to adipocytes, *Journal of*  
946 *Lipid Research* 43 (2002) 676-84.

947 [24] F. Grun, H. Watanabe, Z. Zamanian, L. Maeda, K. Arima, R. Cubacha, D.M. Gardiner, J. Kanno, T.  
948 Iguchi, B. Blumberg, Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in  
949 vertebrates, *Mol Endocrinol* 20(9) (2006) 2141-55.

950 [25] R.M. Sargis, D.N. Johnson, R.A. Choudhury, M.J. Brady, Environmental Endocrine Disruptors  
951 Promote Adipogenesis in the 3T3-L1 Cell Line through Glucocorticoid Receptor Activation, *Obesity*  
952 *(Silver Spring, Md.)* 18(7) (2010) 1283-1288.

953 [26] A. Riu, M. Grimaldi, A. le Maire, G. Bey, K. Phillips, A. Boulahtouf, E. Perdu, D. Zalko, W.  
954 Bourguet, P. Balaguer, Peroxisome proliferator-activated receptor gamma is a target for halogenated  
955 analogs of bisphenol A, *Environmental health perspectives* 119(9) (2011) 1227-32.

956 [27] A. Riu, C.W. McCollum, C.L. Pinto, M. Grimaldi, A. Hillenweck, E. Perdu, D. Zalko, L. Bernard,  
957 V. Laudet, P. Balaguer, M. Bondesson, J.A. Gustafsson, Halogenated bisphenol-A analogs act as  
958 obesogens in zebrafish larvae (*Danio rerio*), *Toxicological sciences : an official journal of the Society of*  
959 *Toxicology* 139(1) (2014) 48-58.

960 [28] X. Li, J. Ycaza, B. Blumberg, The environmental obesogen tributyltin chloride acts via peroxisome  
961 proliferator activated receptor gamma to induce adipogenesis in murine 3T3-L1 preadipocytes, *The*  
962 *Journal of Steroid Biochemistry and Molecular Biology* 127(1-2) (2011) 9-15.

963 [29] R. Chamorro-Garcia, M. Sahu, R.J. Abbey, J. Laude, N. Pham, B. Blumberg, Transgenerational  
964 inheritance of increased fat depot size, stem cell reprogramming, and hepatic steatosis elicited by prenatal  
965 exposure to the obesogen tributyltin in mice, *Environmental health perspectives* 121(3) (2013) 359-66.

966 [30] F.S. Vom Saal, S.C. Nagel, B.L. Coe, B.M. Angle, J.A. Taylor, The estrogenic endocrine disrupting  
967 chemical bisphenol A (BPA) and obesity, *Molecular and cellular endocrinology* 354(1-2) (2012) 74-84.

968 [31] B.M. Angle, R.P. Do, D. Ponzi, R.W. Stahlhut, B.E. Drury, S.C. Nagel, W.V. Welshons, C.L. Besch-  
969 Williford, P. Palanza, S. Parmigiani, F.S. vom Saal, J.A. Taylor, Metabolic disruption in male mice due to  
970 fetal exposure to low but not high doses of bisphenol A (BPA): Evidence for effects on body weight, food  
971 intake, adipocytes, leptin, adiponectin, insulin and glucose regulation, *Reproductive Toxicology* 42  
972 (2013) 256-268.

973 [32] C.D. Kassotis, L. Masse, S. Kim, J.J. Schlezinger, T.F. Webster, H.M. Stapleton, Characterization of  
974 adipogenic chemicals in three different cell culture systems: implications for reproducibility based on cell  
975 source and handling, *Scientific reports* 7 (2017) 42104.

976 [33] K. Zebisch, V. Voigt, M. Wabitsch, M. Brandsch, Protocol for effective differentiation of 3T3-L1  
977 cells to adipocytes, *Anal Biochem* 425(1) (2012) 88-90.

978 [34] A. Kleensang, M.M. Vantangoli, S. Odwin-DaCosta, M.E. Andersen, K. Boekelheide, M. Bouhifd,  
979 A.J. Fornace, Jr., H.H. Li, C.B. Livi, S. Madnick, A. Maertens, M. Rosenberg, J.D. Yager, L. Zhaog, T.  
980 Hartung, Genetic variability in a frozen batch of MCF-7 cells invisible in routine authentication affecting  
981 cell function, *Scientific reports* 6 (2016) 28994.

982 [35] C.D. Kassotis, K. Hoffman, J. Volker, Y. Pu, A. Veiga-Lopez, S.M. Kim, J.J. Schlezinger, P.  
983 Bovolín, E. Cottone, A. Saraceni, R. Scandiffio, E. Atlas, K. Leingartner, S. Krager, S.A. Tischkau, S.  
984 Ermler, J. Legler, V.A. Chappell, S.E. Fenton, F. Mesmar, M. Bondesson, M.F. Fernandez, H.M.  
985 Stapleton, Reproducibility of adipogenic responses to metabolism disrupting chemicals in the 3T3-L1  
pre-adipocyte model system: An interlaboratory study, *Toxicology* 461 (2021) 152900.

987 [36] C.N. Ramskov Tetzlaff, T. Svingen, A.M. Vinggaard, A.K. Rosenmai, C. Taxvig, Bisphenols B, E,  
988 F, and S and 4-cumylphenol induce lipid accumulation in mouse adipocytes similarly to bisphenol A,  
989 *Environ Toxicol* 35(5) (2020) 543-552.

990 [37] C.D. Kassotis, K. Hoffman, H.M. Stapleton, Characterization of Adipogenic Activity of Semi-  
991 volatile Indoor Contaminants and House Dust, *Environmental science & technology* In 51(15) (2017)  
992 8735-8745.

993 [38] L.E. Armstrong, S. Akinbo, A.L. Slitt, 2,2',4,4',5-Pentabromodiphenyl ether induces lipid  
994 accumulation throughout differentiation in 3T3-L1 and human preadipocytes in vitro, *J Biochem Mol*  
995 *Toxicol* 34(6) (2020) e22485.

996 [39] E.W. Tung, A. Boudreau, M.G. Wade, E. Atlas, Induction of adipocyte differentiation by  
997 polybrominated diphenyl ethers (PBDEs) in 3T3-L1 cells, *PLoS one* 9(4) (2014) e94583.

998 [40] J. Xu, P. Shimpi, L. Armstrong, D. Salter, A.L. Slitt, PFOS induces adipogenesis and glucose uptake  
999 in association with activation of Nrf2 signaling pathway, *Toxicology and applied pharmacology* 290  
1000 (2016) 21-30.

1001 [41] A.M. Watkins, C.R. Wood, M.T. Lin, B.D. Abbott, The effects of perfluorinated chemicals on  
1002 adipocyte differentiation in vitro, *Molecular and cellular endocrinology* 400 (2015) 90-101.

1003 [42] Johannes Völker, Felicity Ashcroft, Åsa Vedøy, Lisa Zimmermann, M. Wagner, Adipogenic Activity  
1004 of Chemicals Used in Plastic Consumer Products, *Environmental science & technology* 56 (2022) 2487-  
1005 2496.

1006 [43] F. Ariemma, V. D'Esposito, D. Liguoro, F. Oriente, S. Cabaro, A. Liotti, I. Cimmino, M. Longo, F.  
1007 Beguinot, P. Formisano, R. Valentino, Low-Dose Bisphenol-A Impairs Adipogenesis and Generates  
1008 Dysfunctional 3T3-L1 Adipocytes, *PLoS one* 11(3) (2016) e0150762.

1009 [44] J.M. Lane, J.R. Doyle, J.P. Fortin, A.S. Kopin, J.M. Ordovas, Development of an OP9 derived cell  
1010 line as a robust model to rapidly study adipocyte differentiation, *PLoS one* 9(11) (2014) e112123.

1011 [45] F.V. Andrews, S.M. Kim, L. Edwards, J.J. Schlezinger, Identifying adipogenic chemicals: Disparate  
1012 effects in 3T3-L1, OP9 and primary mesenchymal multipotent cell models, *Toxicology in vitro : an*  
1013 *international journal published in association with BIBRA* 67 (2020) 104904.

1014 [46] I. Zenbio, *Subcutaneous Human Adipocytes Manual*, 2015.

1015 [47] M. Sigma, *Human Preadipocytes (HPAd) Culture Protocol*, 2020.  
1016 <https://www.sigmaaldrich.com/technical-documents/protocols/biology/human-preadipocytes.html>.  
1017 (Accessed 4 November 2020).

1018 [48] J.S. You, Y.J. Lee, K.S. Kim, S.H. Kim, K.J. Chang, Ethanol extract of lotus (*Nelumbo nucifera*)  
1019 root exhibits an anti-adipogenic effect in human pre-adipocytes and anti-obesity and anti-oxidant effects  
1020 in rats fed a high-fat diet, *Nutr Res* 34(3) (2014) 258-67.

1021 [49] J.S. You, Y.J. Lee, K.S. Kim, S.H. Kim, K.J. Chang, Anti-obesity and hypolipidaemic effects of  
1022 *Nelumbo nucifera* seed ethanol extract in human pre-adipocytes and rats fed a high-fat diet, *J Sci Food*  
1023 *Agric* 94(3) (2014) 568-75.

1024 [50] L. Li, S.J. Lee, S.Y. Kook, T.G. Ahn, J.Y. Lee, J.Y. Hwang, Serum from pregnant women with  
1025 gestational diabetes mellitus increases the expression of FABP4 mRNA in primary subcutaneous human  
1026 pre-adipocytes, *Obstet Gynecol Sci* 60(3) (2017) 274-282.

1027 [51] V. Peshdary, G. Styles, R. Gagne, C.L. Yauk, A. Sorisky, E. Atlas, Depot-Specific Analysis of  
1028 Human Adipose Cells and Their Responses to Bisphenol S, *Endocrinology* 161(6) (2020).

1029 [52] M. Wabitsch, R.E. Brenner, I. Melzner, M. Braun, P. Moller, E. Heinze, K.M. Debatin, H. Hauner,  
1030 Characterization of a human preadipocyte cell strain with high capacity for adipose differentiation, *Int J*  
1031 *Obes Relat Metab Disord* 25(1) (2001) 8-15.

1032 [53] P. Fischer-Posovszky, F.S. Newell, M. Wabitsch, H.E. Tornqvist, Human SGBS cells - a unique tool  
1033 for studies of human fat cell biology, *Obes Facts* 1(4) (2008) 184-9.

1034 [54] E.H. Allott, E. Oliver, J. Lysaght, S.G. Gray, J.V. Reynolds, H.M. Roche, G.P. Pidgeon, The SGBS  
1035 cell strain as a model for the in vitro study of obesity and cancer, *Clin Transl Oncol* 14 (2012) 9.

1036 [55] A. Guennoun, M. Kazantzis, R. Thomas, M. Wabitsch, D. Tews, K. Seetharama Sastry, M.  
1037 Abdelkarim, V. Zilberfarb, A.D. Strosberg, L. Chouchane, Comprehensive molecular characterization of  
1038 human adipocytes reveals a transient brown phenotype, *J Transl Med* 13 (2015) 135.  
1039 [56] T. Montanari, M. Colitti, Simpson-Golabi-Behmel syndrome human adipocytes reveal a changing  
1040 phenotype throughout differentiation, *Histochem Cell Biol* 149(6) (2018) 593-605.  
1041 [57] C.R. Yeo, M. Agrawal, S. Hoon, A. Shabbir, M.K. Shrivastava, S. Huang, C.M. Khoo, V. Chhay,  
1042 M.S. Yassin, E.S. Tai, A. Vidal-Puig, S.A. Toh, SGBS cells as a model of human adipocyte browning: A  
1043 comprehensive comparative study with primary human white subcutaneous adipocytes, *Scientific reports*  
1044 7(1) (2017) 4031.  
1045 [58] S. Kalkhof, L. Krieg, P. Buttner, M. Wabitsch, C. Kuntzel, D. Friebe, K. Landgraf, M. Hanschkow,  
1046 K. Schubert, W. Kiess, K. Krohn, M. Bluher, M. von Bergen, A. Korner, In *Depth Quantitative Proteomic*  
1047 *and Transcriptomic Characterization of Human Adipocyte Differentiation Using the SGBS Cell Line,*  
1048 *Proteomics* (2020) e1900405.  
1049 [59] F. Miehle, G. Moller, A. Cecil, J. Lintelmann, M. Wabitsch, J. Tokarz, J. Adamski, M. Haid,  
1050 Lipidomic Phenotyping Reveals Extensive Lipid Remodeling during Adipogenesis in Human Adipocytes,  
1051 *Metabolites* 10(6) (2020).  
1052 [60] A. Schaffert, L. Krieg, J. Weiner, R. Schlichting, E. Ueberham, I. Karkossa, M. Bauer, K. Landgraf,  
1053 K.M. Junge, M. Wabitsch, J. Lehmann, B.I. Escher, A.C. Zenclussen, A. Korner, M. Bluher, J.T. Heiker,  
1054 M. von Bergen, K. Schubert, Alternatives for the worse: Molecular insights into adverse effects of  
1055 bisphenol a and substitutes during human adipocyte differentiation, *Environment international* 156 (2021)  
1056 106730.  
1057 [61] M. Kazantzis, V. Takahashi, J. Hinkle, S. Kota, V. Zilberfarb, T. Issad, M. Abdelkarim, L.  
1058 Chouchane, A.D. Strosberg, PAZ6 cells constitute a representative model for human brown pre-  
1059 adipocytes, *Front Endocrinol (Lausanne)* 3 (2012) 13.  
1060 [62] A.S. Janesick, G. Dimastrogiovanni, L. Vanek, C. Boulos, R. Chamorro-García, W. Tang, B.  
1061 Blumberg, On the Utility of ToxCast and ToxPi as Methods for Identifying New Obesogens,  
1062 *Environmental health perspectives* 124(8) (2016) 1214-1226.  
1063 [63] B.M. Shoucri, E.S. Martinez, T.J. Abreo, V.T. Hung, Z. Moosova, T. Shioda, B. Blumberg, Retinoid  
1064 X Receptor Activation Alters the Chromatin Landscape To Commit Mesenchymal Stem Cells to the  
1065 Adipose Lineage, *Endocrinology* 158(10) (2017) 3109-3125.  
1066 [64] M.E. Bateman, A.L. Strong, J.A. McLachlan, M.E. Burow, B.A. Bunnell, The Effects of Endocrine  
1067 Disruptors on Adipogenesis and Osteogenesis in Mesenchymal Stem Cells: A Review, *Front Endocrinol*  
1068 *(Lausanne)* 7 (2016) 171.  
1069 [65] Q.Q. Tang, T.C. Otto, M.D. Lane, Commitment of C3H10T1/2 pluripotent stem cells to the  
1070 adipocyte lineage, *Proceedings of the National Academy of Sciences of the United States of America*  
1071 101(26) (2004) 9607-11.  
1072 [66] T. Katagiri, A. Yamaguchi, T. Ikeda, S. Yoshiki, J.M. Wozney, V. Rosen, E.A. Wang, H. Tanaka, S.  
1073 Omura, T. Suda, The non-osteogenic mouse pluripotent cell line, C3H10T1/2, is induced to differentiate  
1074 into osteoblastic cells by recombinant human bone morphogenetic protein-2, *Biochem Biophys Res*  
1075 *Commun* 172(1) (1990) 295-9.  
1076 [67] S. Muruganandan, A.A. Roman, C.J. Sinal, Adipocyte differentiation of bone marrow-derived  
1077 mesenchymal stem cells: cross talk with the osteoblastogenic program, *Cellular and molecular life*  
1078 *sciences : CMLS* 66(2) (2009) 236-53.  
1079 [68] R. Yue, Bo O. Zhou, Issei S. Shimada, Z. Zhao, Sean J. Morrison, Leptin Receptor Promotes  
1080 Adipogenesis and Reduces Osteogenesis by Regulating Mesenchymal Stromal Cells in Adult Bone  
1081 Marrow, *Cell Stem Cell* 18(6) (2016) 782-796.  
1082 [69] C. Csaki, U. Matis, A. Mobasheri, H. Ye, M. Shakibaei, Chondrogenesis, osteogenesis and  
1083 adipogenesis of canine mesenchymal stem cells: a biochemical, morphological and ultrastructural study,  
1084 *Histochem Cell Biol* 128(6) (2007) 507-20.  
1085 [70] S. Kim, A. Li, S. Monti, J.J. Schlezinger, Tributyltin induces a transcriptional response without a  
1086 brite adipocyte signature in adipocyte models, *Archives of toxicology* (2018).

1087 [71] A.H. Baker, J. Watt, C.K. Huang, L.C. Gerstenfeld, J.J. Schlezinger, Tributyltin engages multiple  
1088 nuclear receptor pathways and suppresses osteogenesis in bone marrow multipotent stromal cells,  
1089 *Chemical research in toxicology* 28(6) (2015) 1156-66.  
1090 [72] R. Chamorro-Garcia, S. Kirchner, X. Li, A. Janesick, S.C. Casey, C. Chow, B. Blumberg, Bisphenol  
1091 A diglycidyl ether induces adipogenic differentiation of multipotent stromal stem cells through a  
1092 peroxisome proliferator-activated receptor gamma-independent mechanism, *Environmental health*  
1093 *perspectives* 120(7) (2012) 984-9.  
1094 [73] I. Reina-Perez, A. Olivas-Martinez, V. Mustieles, F.J. Ruiz-Ojeda, J.M. Molina-Molina, N. Olea,  
1095 M.F. Fernandez, Bisphenol F and bisphenol S promote lipid accumulation and adipogenesis in human  
1096 adipose-derived stem cells, *Food and chemical toxicology : an international journal published for the*  
1097 *British Industrial Biological Research Association* 152 (2021) 112216.  
1098 [74] I.C. Cohen, E.R. Cohenour, K.G. Harnett, S.M. Schuh, BPA, BPAF and TMBPF Alter Adipogenesis  
1099 and Fat Accumulation in Human Mesenchymal Stem Cells, with Implications for Obesity, *Int J Mol Sci*  
1100 22(10) (2021).  
1101 [75] K. Norgren, A. Tuck, A. Vieira Silva, P. Burkhardt, M. Oberg, V. Munic Kos, High throughput  
1102 screening of bisphenols and their mixtures under conditions of low-intensity adipogenesis of human  
1103 mesenchymal stem cells (hMSCs), *Food and chemical toxicology : an international journal published for*  
1104 *the British Industrial Biological Research Association* 161 (2022) 112842.  
1105 [76] P. Hu, H. Overby, E. Heal, S. Wang, J. Chen, C.L. Shen, L. Zhao, Methylparaben and butylparaben  
1106 alter multipotent mesenchymal stem cell fates towards adipocyte lineage, *Toxicology and applied*  
1107 *pharmacology* 329 (2017) 48-57.  
1108 [77] B.M. Shoucri, V.T. Hung, R. Chamorro-Garcia, T. Shioda, B. Blumberg, Retinoid X Receptor  
1109 Activation During Adipogenesis of Female Mesenchymal Stem Cells Programs a Dysfunctional  
1110 Adipocyte, *Endocrinology* 159(8) (2018) 2863-2883.  
1111 [78] S.C. Yanik, A.H. Baker, K.K. Mann, J.J. Schlezinger, Organotins are potent activators of  
1112 PPARgamma and adipocyte differentiation in bone marrow multipotent mesenchymal stromal cells,  
1113 *Toxicological sciences : an official journal of the Society of Toxicology* 122(2) (2011) 476-88.  
1114 [79] C.D. Kassotis, M. LeFauve, Y.-T.T. Chiang, M.N. Knuth, S. Schkoda, S.W. Kullman, Nonylphenol  
1115 Polyethoxylates Enhance Adipose Deposition in Developmentally Exposed Zebrafish, *Toxics* 10(99)  
1116 (2022).  
1117 [80] M.J. Kim, V. Pelloux, E. Guyot, J. Tordjman, L.C. Bui, A. Chevallier, C. Forest, C. Benelli, K.  
1118 Clement, R. Barouki, Inflammatory pathway genes belong to major targets of persistent organic pollutants  
1119 in adipose cells, *Environmental health perspectives* 120(4) (2012) 508-14.  
1120 [81] B. Foley, D.L. Doheny, M.B. Black, S.N. Pendse, B.A. Wetmore, R.A. Clewell, M.E. Andersen, C.  
1121 Deisenroth, Screening ToxCast Prioritized Chemicals for PPARG Function in a Human Adipose-Derived  
1122 Stem Cell Model of Adipogenesis, *Toxicological sciences : an official journal of the Society of*  
1123 *Toxicology* 155(1) (2017) 85-100.  
1124 [82] W. Wang, K. Itaka, S. Ohba, N. Nishiyama, U.I. Chung, Y. Yamasaki, K. Kataoka, 3D spheroid  
1125 culture system on micropatterned substrates for improved differentiation efficiency of multipotent  
1126 mesenchymal stem cells, *Biomaterials* 30(14) (2009) 2705-15.  
1127 [83] P.R. Baraniak, T.C. McDevitt, Scaffold-free culture of mesenchymal stem cell spheroids in  
1128 suspension preserves multilineage potential, *Cell Tissue Res* 347(3) (2012) 701-11.  
1129 [84] N.C. Cheng, S. Wang, T.H. Young, The influence of spheroid formation of human adipose-derived  
1130 stem cells on chitosan films on stemness and differentiation capabilities, *Biomaterials* 33(6) (2012) 1748-  
1131 58.  
1132 [85] P.A. Turner, B. Gurumurthy, J.L. Bailey, C.M. Elks, A.V. Janorkar, Adipogenic Differentiation of  
1133 Human Adipose-Derived Stem Cells Grown as Spheroids, *Process Biochem* 59 (2017) 312-320.  
1134 [86] P.A. Turner, L.M. Harris, C.A. Purser, R.C. Baker, A.V. Janorkar, A surface-tethered spheroid  
1135 model for functional evaluation of 3T3-L1 adipocytes, *Biotechnol Bioeng* 111(1) (2014) 174-83.



1136 [87] A. Ioannidou, S. Alatar, R. Schipper, F. Baganha, M. Ahlander, A. Hornell, R.M. Fisher, C.E.  
1137 Hagberg, Hypertrophied human adipocyte spheroids as in vitro model of weight gain and adipose tissue  
1138 dysfunction, *J Physiol* (2021).  
1139 [88] Y. Miyagawa, H. Okita, M. Hiroyama, R. Sakamoto, M. Kobayashi, H. Nakajima, Y.U. Katagiri, J.  
1140 Fujimoto, J. Hata, A. Umezawa, N. Kiyokawa, A microfabricated scaffold induces the spheroid formation  
1141 of human bone marrow-derived mesenchymal progenitor cells and promotes efficient adipogenic  
1142 differentiation, *Tissue Eng Part A* 17(3-4) (2011) 513-21.  
1143 [89] S.K. Kapur, X. Wang, H. Shang, S. Yun, X. Li, G. Feng, M. Khurgel, A.J. Katz, Human adipose  
1144 stem cells maintain proliferative, synthetic and multipotential properties when suspension cultured as self-  
1145 assembling spheroids, *Biofabrication* 4(2) (2012) 025004.  
1146 [90] A. Ioannidou, S. Alatar, R. Schipper, F. Baganha, M. Ahlander, A. Hornell, R.M. Fisher, C.E.  
1147 Hagberg, Hypertrophied human adipocyte spheroids as in vitro model of weight gain and adipose tissue  
1148 dysfunction, *J Physiol* 600(4) (2022) 869-883.  
1149 [91] P.A. Turner, Y. Tang, S.J. Weiss, A.V. Janorkar, Three-dimensional spheroid cell model of in vitro  
1150 adipocyte inflammation, *Tissue Eng Part A* 21(11-12) (2015) 1837-47.  
1151 [92] A.J. Klingelhutz, F.A. Gourronc, A. Chaly, D.A. Wadkins, A.J. Burand, K.R. Markan, S.O. Idiga, M.  
1152 Wu, M.J. Potthoff, J.A. Ankrum, Scaffold-free generation of uniform adipose spheroids for metabolism  
1153 research and drug discovery, *Scientific reports* 8(1) (2018) 523.  
1154 [93] J.X. Shen, M. Couchet, J. Dufau, T. de Castro Barbosa, M.H. Ulbrich, M. Helmstadter, A.M. Kemas,  
1155 R. Zandi Shafagh, M.A. Marques, J.B. Hansen, N. Mejhert, D. Langin, M. Ryden, V.M. Lauschke, 3D  
1156 Adipose Tissue Culture Links the Organotypic Microenvironment to Improved Adipogenesis, *Adv Sci*  
1157 (Weinh) 8(16) (2021) e2100106.  
1158 [94] F.A. Müller, S.J. Sturla, Human in vitro models of nonalcoholic fatty liver disease, *Current Opinion*  
1159 *in Toxicology* 16 (2019) 9-16.  
1160 [95] N.C. Chavez-Tapia, N. Rosso, C. Tiribelli, In vitro models for the study of non-alcoholic fatty liver  
1161 disease, *Current medicinal chemistry* 18(7) (2011) 1079-84.  
1162 [96] G. Kanuri, I. Bergheim, In vitro and in vivo models of non-alcoholic fatty liver disease (NAFLD), *Int*  
1163 *J Mol Sci* 14(6) (2013) 11963-80.  
1164 [97] P.A. Soret, J. Magusto, C. Housset, J. Gautheron, In Vitro and In Vivo Models of Non-Alcoholic  
1165 Fatty Liver Disease: A Critical Appraisal, *J Clin Med* 10(1) (2020).  
1166 [98] L. Peyre, P. Rouimi, G. de Sousa, C. Helies-Toussaint, B. Carre, S. Barcellini, M.C. Chagnon, R.  
1167 Rahmani, Comparative study of bisphenol A and its analogue bisphenol S on human hepatic cells: a focus  
1168 on their potential involvement in nonalcoholic fatty liver disease, *Food and chemical toxicology : an*  
1169 *international journal published for the British Industrial Biological Research Association* 70 (2014) 9-18.  
1170 [99] R. Cano, J.L. Perez, L.A. Davila, A. Ortega, Y. Gomez, N.J. Valero-Cedeno, H. Parra, A. Manzano,  
1171 T.I. Veliz Castro, M.P.D. Albornoz, G. Cano, J. Rojas-Quintero, M. Chacin, V. Bermudez, Role of  
1172 Endocrine-Disrupting Chemicals in the Pathogenesis of Non-Alcoholic Fatty Liver Disease: A  
1173 Comprehensive Review, *Int J Mol Sci* 22(9) (2021).  
1174 [100] H. Chuntao, Y. Xiao, F. Huiling, Z. Xi, Z. Jing, M. Bixian, Yang, Y. Yunjiang, TPhP Induced Lipid  
1175 Metabolism Disruption Mediated by miRNA in Hepatocyte, 2008 (2021) 140-150.  
1176 [101] P. Cocci, G. Mosconi, F.A. Palermo, Changes in expression of microRNA potentially targeting key  
1177 regulators of lipid metabolism in primary gilthead sea bream hepatocytes exposed to phthalates or flame  
1178 retardants, *Aquat Toxicol* 209 (2019) 81-90.  
1179 [102] J.S. Yang, W. Qi, R. Farias-Pereira, S. Choi, J.M. Clark, D. Kim, Y. Park, Permethrin and  
1180 ivermectin modulate lipid metabolism in steatosis-induced HepG2 hepatocyte, *Food and chemical*  
1181 *toxicology : an international journal published for the British Industrial Biological Research Association*  
1182 125 (2019) 595-604.  
1183 [103] S. Rose, M. Cuvellier, F. Ezan, J. Carteret, A. Bruyere, V. Legagneux, F. Nesslany, G. Baffet, S.  
1184 Langouet, DMSO-free highly differentiated HepaRG spheroids for chronic toxicity, liver functions and  
1185 genotoxicity studies, *Archives of toxicology* (2021).

1186 [104] M.T. Donato, L. Tolosa, M.J. Gomez-Lechon, Culture and Functional Characterization of Human  
1187 Hepatoma HepG2 Cells, *Methods Mol Biol* 1250 (2015) 77-93.  
1188 [105] L. Huc, A. Lemarie, F. Gueraud, C. Helies-Toussaint, Low concentrations of bisphenol A induce  
1189 lipid accumulation mediated by the production of reactive oxygen species in the mitochondria of HepG2  
1190 cells, *Toxicology in vitro : an international journal published in association with BIBRA* 26(5) (2012)  
1191 709-17.  
1192 [106] H.R. Yao, J. Liu, D. Plumeri, Y.B. Cao, T. He, L. Lin, Y. Li, Y.Y. Jiang, J. Li, J. Shang,  
1193 Lipotoxicity in HepG2 cells triggered by free fatty acids, *Am J Transl Res* 3(3) (2011) 284-91.  
1194 [107] S. Sefried, H.U. Haring, C. Weigert, S.S. Eckstein, Suitability of hepatocyte cell lines HepG2,  
1195 AML12 and THLE-2 for investigation of insulin signalling and hepatokine gene expression, *Open Biol*  
1196 8(10) (2018).  
1197 [108] A. Herms, M. Bosch, N. Ariotti, B.J. Reddy, A. Fajardo, A. Fernandez-Vidal, A. Alvarez-Guaita,  
1198 M.A. Fernandez-Rojo, C. Rentero, F. Tebar, C. Enrich, M.I. Geli, R.G. Parton, S.P. Gross, A. Pol, Cell-  
1199 to-cell heterogeneity in lipid droplets suggests a mechanism to reduce lipotoxicity, *Curr Biol* 23(15)  
1200 (2013) 1489-96.  
1201 [109] C. Aninat, A. Piton, D. Glaise, T.L. Charpentier, S. Langouët, F. Morel, C. Guguen-Guillouzo, A.  
1202 Guillouzo, EXPRESSION OF CYTOCHROMES P450, CONJUGATING ENZYMES AND NUCLEAR  
1203 RECEPTORS IN HUMAN HEPATOMA HepaRG CELLS, *Drug Metabolism and Disposition* 34(1)  
1204 (2006) 75-83  
1205 [110] M.V. Brown, S.A. Compton, M.V. Milburn, K.A. Lawton, B. Cheatham, Metabolomic signatures in  
1206 lipid-loaded HepaRGs reveal pathways involved in steatotic progression, *Obesity (Silver Spring, Md.)*  
1207 21(12) (2013) E561-E570  
1208 [111] Y. Wei, D. Wang, M.J. Pagliassotti, Saturated fatty acid-mediated endoplasmic reticulum stress and  
1209 apoptosis are augmented by trans-10, cis-12-conjugated linoleic acid in liver cells, *Molecular and Cellular*  
1210 *Biochemistry* 303(1) (2007) 105-113  
1211 [112] J.M. Adams, T. Pratipanawat, R. Berria, E. Wang, R.A. DeFronzo, M.C. Sullards, L.J. Mandarino,  
1212 Ceramide content is increased in skeletal muscle from obese insulin-resistant humans, *Diabetes* 53(1)  
1213 (2004) 25-31.  
1214 [113] B. Wahlang, J. Jin, J.I. Beier, J.E. Hardesty, E.F. Daly, R.D. Schnegelberger, K.C. Falkner, R.A.  
1215 Prough, I.A. Kirpich, M.C. Cave, Mechanisms of Environmental Contributions to Fatty Liver Disease,  
1216 *Current environmental health reports* 6(3) (2019) 80-94.  
1217 [114] S. Bucher, A. Tête, N. Podechard, M. Liamin, D. Le Guillou, M. Chevanne, C. Coulouarn, M.  
1218 Imran, I. Gallais, M. Fernier, Q. Hamdaoui, M.-A. Robin, O. Sergent, B. Fromenty, D. Lagadic-  
1219 Gossmann, Co-exposure to benzo[a]pyrene and ethanol induces a pathological progression of liver  
1220 steatosis in vitro and in vivo, *Scientific reports* 8(1) (2018) 5963.  
1221 [115] A.L. Roe, G. Howard, R. Blouin, J.E. Snawder, Characterization of cytochrome P450 and  
1222 glutathione S-transferase activity and expression in male and female ob/ob mice, *International Journal of*  
1223 *Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of*  
1224 *Obesity* 23(1) (1999) 48-53.  
1225 [116] D. Zhang, Z.-X. Liu, C.S. Choi, L. Tian, R. Kibbey, J. Dong, G.W. Cline, P.A. Wood, G.I.  
1226 Shulman, Mitochondrial dysfunction due to long-chain Acyl-CoA dehydrogenase deficiency causes  
1227 hepatic steatosis and hepatic insulin resistance, *Proceedings of the National Academy of Sciences of the*  
1228 *United States of America* 104(43) (2007) 17075-17080  
1229 [117] J. Fischer, L. Koch, C. Emmerling, J. Vierkotten, T. Peters, J.C. Brüning, U. Rüther, Inactivation of  
1230 the Fto gene protects from obesity, *Nature* 458(7240) (2009) 894-898  
1231 [118] Y. Zhou, J.X. Shen, V.M. Lauschke, Comprehensive Evaluation of Organotypic and  
1232 Microphysiological Liver Models for Prediction of Drug-Induced Liver Injury, *Frontiers in pharmacology*  
1233 10 (2019) 1093.  
1234 [119] Y. Takahashi, Y. Hori, T. Yamamoto, T. Urashima, Y. Ohara, H. Tanaka, 3D spheroid cultures  
1235 improve the metabolic gene expression profiles of HepaRG cells, *Bioscience Reports* 35(3) (2015)  
1236 e00208.

1237 [120] S. Rose, M. Cuvellier, F. Ezan, J. Carteret, A. Bruyere, V. Legagneux, F. Nessler, G. Baffet, S.  
1238 Langouet, DMSO-free highly differentiated HepaRG spheroids for chronic toxicity, liver functions and  
1239 genotoxicity studies, *Archives of toxicology* 96(1) (2022) 243-258.

1240 [121] A. Guillouzo, F. Morel, S. Langouet, K. Maheo, M. Rissel, Use of hepatocyte cultures for the study  
1241 of hepatotoxic compounds, *J Hepatol* 26 Suppl 2 (1997) 73-80.

1242 [122] A. Guillouzo, A. Corlu, C. Aninat, D. Glaise, F. Morel, C. Guguen-Guillouzo, The human  
1243 hepatoma HepaRG cells: A highly differentiated model for studies of liver metabolism and toxicity of  
1244 xenobiotics, *Chemico-Biological Interactions* 168(1) (2007) 66-73

1245 [123] S. Rose, F. Ezan, M. Cuvellier, A. Bruyère, V. Legagneux, S. Langouët, G. Baffet, Generation of  
1246 proliferating human adult hepatocytes using optimized 3D culture conditions, *Scientific reports* 11 (2021).

1247 [124] S.C. Ramaiahgari, S. Waidyanatha, D. Dixon, M.J. DeVito, R.S. Paules, S.S. Ferguson, Three-  
1248 Dimensional (3D) HepaRG Spheroid Model With Physiologically Relevant Xenobiotic Metabolism  
1249 Competence and Hepatocyte Functionality for Liver Toxicity Screening, *Toxicological Sciences* 160(1)  
1250 (2017) 189-190.

1251 [125] M. Duriez, A. Jacquet, L. Hoet, S. Roche, M.D. Bock, C. Rocher, G. Haussy, X. Vige, Z. Bocskei,  
1252 T. Slavnic, V. Martin, J.C. Guillemot, M. Didier, A. Kannt, C. Orsini, V. Mikol, A.L. Fevre, A 3D  
1253 Human Liver Model of Nonalcoholic Steatohepatitis, *J Clin Transl Hepatol* 8(4) (2020) 359-370.

1254 [126] M. Severgnini, J. Sherman, A. Sehgal, N.K. Jayaprakash, J. Aubin, G. Wang, L. Zhang, C.G. Peng,  
1255 K. Yucius, J. Butler, K. Fitzgerald, A rapid two-step method for isolation of functional primary mouse  
1256 hepatocytes: cell characterization and asialoglycoprotein receptor based assay development,  
1257 *Cytotechnology* 64(2) (2012) 187-95.

1258 [127] S. Yang, A. Zhang, T. Li, R. Gao, C. Peng, L. Liu, Q. Cheng, M. Mei, Y. Song, X. Xiang, C. Wu,  
1259 X. Xiao, Q. Li, Dysregulated Autophagy in Hepatocytes Promotes Bisphenol A-Induced Hepatic Lipid  
1260 Accumulation in Male Mice, *Endocrinology* 158(9) (2017) 2799-2812.

1261 [128] X. Guo, H. Li, H. Xu, V. Halim, W. Zhang, H. Wang, K.T. Ong, S.L. Woo, R.L. Walzem, D.G.  
1262 Mashek, H. Dong, F. Lu, L. Wei, Y. Huo, C. Wu, Palmitoleate induces hepatic steatosis but suppresses  
1263 liver inflammatory response in mice, *PLoS one* 7(6) (2012) e39286.

1264 [129] C.A. Galloway, H. Lee, P.S. Brookes, Y. Yoon, Decreasing mitochondrial fission alleviates hepatic  
1265 steatosis in a murine model of nonalcoholic fatty liver disease, *Am J Physiol Gastrointest Liver Physiol*  
1266 307(6) (2014) G632-41.

1267 [130] G.Y. Go, S.J. Lee, A. Jo, J.R. Lee, J.S. Kang, M. Yang, G.U. Bae, Bisphenol A and estradiol  
1268 impede myoblast differentiation through down-regulating Akt signaling pathway, *Toxicol Lett* 292 (2018)  
1269 12-19.

1270 [131] A.L.F. Davis, A.A. Thomas, K.S. Shorter, S.L. Brown, B.L. Baumgarner, Cellular fatty acid level  
1271 regulates the effect of tolylfluanid on mitochondrial dysfunction and insulin sensitivity in C2C12 skeletal  
1272 myotubes, *Biochemical and biophysical research communications* 505(2) (2018) 392-398.

1273 [132] A.M. Abdelmoez, L. Sardón Puig, J.A.B. Smith, B.M. Gabriel, M. Savikj, L. Dollet, A.V. Chibalin,  
1274 A. Krook, J.R. Zierath, N.J. Pilon, Comparative profiling of skeletal muscle models reveals heterogeneity  
1275 of transcriptome and metabolism, *Am J Physiol Cell Physiol* 318(3) (2020) C615-C626.

1276 [133] P. Rajesh, K. Balasubramanian, Di(2-ethylhexyl)phthalate exposure impairs insulin receptor and  
1277 glucose transporter 4 gene expression in L6 myotubes, *Hum Exp Toxicol* 33(7) (2014) 685-700.

1278 [134] V.K. Singh, S.K. Sarkar, A. Saxena, B.C. Koner, Effect of Subtoxic DDT Exposure on Glucose  
1279 Uptake and Insulin Signaling in Rat L6 Myoblast-Derived Myotubes, *International journal of toxicology*  
1280 38(4) (2019) 303-311.

1281 [135] D. Coletti, S. Palleschi, L. Silvestroni, A. Cannavò, E. Vivarelli, F. Tomei, M. Molinaro, S. Adamo,  
1282 Polychlorobiphenyls inhibit skeletal muscle differentiation in culture, *Toxicol Appl Pharmacol* 175(3)  
1283 (2001) 226-33.

1284 [136] R. Zhang, I.N. Pessah, Divergent Mechanisms Leading to Signaling Dysfunction in Embryonic  
1285 Muscle by Bisphenol A and Tetrabromobisphenol A, *Mol Pharmacol* 91(4) (2017) 428-436.

1286 [137] V. Aas, S.S. Bakke, Y.Z. Feng, E.T. Kase, J. Jensen, S. Bajpeyi, G.H. Thoresen, A.C. Rustan, Are  
1287 cultured human myotubes far from home?, *Cell Tissue Res* 354(3) (2013) 671-82.

1288 [138] T.B. Hayes, L.L. Anderson, V.R. Beasley, S.R. de Solla, T. Iguchi, H. Ingraham, P. Kestemont, J.  
1289 Kniewald, Z. Kniewald, V.S. Langlois, E.H. Luque, K.A. McCoy, M. Munoz-de-Toro, T. Oka, C.A.  
1290 Oliveira, F. Orton, S. Ruby, M. Suzawa, L.E. Tavera-Mendoza, V.L. Trudeau, A.B. Victor-Costa, E.  
1291 Willingham, Demasculinization and feminization of male gonads by atrazine: consistent effects across  
1292 vertebrate classes, *J Steroid Biochem Mol Biol* 127(1-2) (2011) 64-73.

1293 [139] A. Capitão, A. Lyssimachou, L.F.C. Castro, M.M. Santos, Obesogens in the aquatic environment:  
1294 an evolutionary and toxicological perspective, *Environ Int* 106 (2017) 153-169.

1295 [140] M. Kleinert, C. Clemmensen, S.M. Hofmann, M.C. Moore, S. Renner, S.C. Woods, P. Huypens, J.  
1296 Beckers, M.H. de Angelis, A. Schürmann, M. Bakhti, M. Klingenspor, M. Heiman, A.D. Cherrington, M.  
1297 Ristow, H. Lickert, E. Wolf, P.J. Havel, T.D. Müller, M.H. Tschöp, Animal models of obesity and  
1298 diabetes mellitus, *Nat Rev Endocrinol* 14(3) (2018) 140-162.

1299 [141] A. Schlegel, D.Y.R. Stainier, Lessons from “Lower” Organisms: What Worms, Flies, and Zebrafish  
1300 Can Teach Us about Human Energy Metabolism, *PLoS genetics* 3(11) (2007) e199.

1301 [142] K. Benchoula, A. Khatib, A. Jaffar, Q.U. Ahmed, W.M.A.W. Sulaiman, R.A. Wahab, H.R. El-  
1302 Seedi, The promise of zebrafish as a model of metabolic syndrome, *Experimental animals* 68(4) (2019)  
1303 407-416.

1304 [143] K. Howe, M.D. Clark, C.F. Torroja, J. Tarrance, C. Berthelot, M. Muffato, J.E. Collins, S.  
1305 Humphray, K. McLaren, L. Matthews, S. McLaren, I. Sealy, M. Caccamo, C. Churcher, C. Scott, J.C.  
1306 Barrett, R. Koch, G.-J. Rauch, S. White, W. Chow, B. Kilian, L.T. Quintais, J.A. Guerra-Assunção, Y.  
1307 Zhou, Y. Gu, J. Yen, J.-H. Vogel, T. Eyre, S. Redmond, R. Banerjee, J. Chi, B. Fu, E. Langley, S.F.  
1308 Maguire, G.K. Laird, D. Lloyd, E. Kenyon, S. Donaldson, H. Sehra, J. Almeida-King, J. Loveland, S.  
1309 Trevanion, M. Jones, M. Quail, D. Willey, A. Hunt, J. Burton, S. Sims, K. McLay, B. Plumb, J. Davis, C.  
1310 Clee, K. Oliver, R. Clark, C. Riddle, D. Elliot, D. Elliott, G. Threadgold, G. Harden, D. Ware, S. Begum,  
1311 B. Mortimore, B. Mortimer, G. Kerry, P. Heath, B. Phillipmore, A. Tracey, N. Corby, M. Dunn, C.  
1312 Johnson, J. Wood, S. Clark, S. Pelan, G. Griffiths, M. Smith, R. Glithero, P. Howden, N. Barker, C.  
1313 Lloyd, C. Stevens, J. Harley, K. Holt, G. Panagiotidis, J. Lovell, H. Beasley, C. Henderson, D. Gordon,  
1314 K. Auger, D. Wright, J. Collins, C. Raisen, L. Dyer, K. Leung, L. Robertson, K. Ambridge, D.  
1315 Leongamornlert, S. McGuire, R. Gilderthorp, C. Griffiths, D. Manthravadi, S. Nichol, G. Barker, S.  
1316 Whitehead, M. Kay, J. Brown, C. Murnane, E. Gray, M. Humphries, N. Sycamore, D. Barker, D.  
1317 Saunders, J. Wallis, A. Babbage, S. Hammond, M. Mashreghi-Mohammadi, L. Barr, S. Martin, P. Wray,  
1318 A. Ellington, N. Matthews, M. Ellwood, R. Woodmansey, G. Clark, J.D. Cooper, J. Cooper, A. Tromans,  
1319 D. Grafham, C. Skuce, R. Pandian, R. Andrews, E. Harrison, A. Kimberley, J. Garnett, N. Fosker, R.  
1320 Hall, P. Garner, D. Kelly, C. Bird, S. Palmer, I. Gehring, A. Berger, C.M. Dooley, Z. Ersan-Ürün, C.  
1321 Eser, H. Geiger, M. Geisler, L. Karotki, A. Kirn, J. Konantz, M. Konantz, M. Oberländer, S. Rudolph-  
1322 Geiger, M. Teucke, C. Lanz, G. Raddatz, K. Osoegawa, B. Zhu, A. Rapp, S. Widaa, C. Langford, F.  
1323 Yang, S.C. Schuster, N.P. Carter, J. Harrow, Z. Ning, J. Herrero, S.M.J. Searle, A. Enright, R. Geisler,  
1324 R.H.A. Plasterk, C. Lee, M. Westerfield, P.J. de Jong, L.I. Zon, J.H. Postlethwait, C. Nüsslein-Volhard,  
1325 T.J.P. Hubbard, H. Roest Crollius, J. Rogers, D.L. Stemple, The zebrafish reference genome sequence  
1326 and its relationship to the human genome, *Nature* 496(7446) (2013) 498-503.

1327 [144] P. Shen, Y. Yue, J. Zheng, Y. Park, *Caenorhabditis elegans*: A Convenient In Vivo Model for  
1328 Assessing the Impact of Food Bioactive Compounds on Obesity, Aging, and Alzheimer's Disease, *Annu*  
1329 *Rev Food Sci Technol* 9 (2018) 1-22.

1330 [145] H. Volkoff, Fish as models for understanding the vertebrate endocrine regulation of feeding and  
1331 weight, *Molecular and Cellular Endocrinology* 497 (2019) 110437.

1332 [146] C.G. Warr, K.H. Shaw, A. Azim, M.D.W. Piper, L.M. Parsons, Using Mouse and *Drosophila*  
1333 Models to Investigate the Mechanistic Links between Diet, Obesity, Type II Diabetes, and Cancer,  
1334 *International journal of molecular sciences* 19(12) (2018).

1335 [147] G.J. Lieschke, P.D. Currie, Animal models of human disease: zebrafish swim into view, *Nature*  
1336 *Reviews. Genetics* 8(5) (2007) 353-367

1337 [148] T. Teame, Z. Zhang, C. Ran, H. Zhang, Y. Yang, Q. Ding, M. Xie, C. Gao, Y. Ye, M. Duan, Z.  
1338 Zhou, The use of zebrafish (*Danio rerio*) as biomedical models, *Animal Frontiers* 9(3) (2019) 68-77.

- 1339 [149] J.R. Goldsmith, C. Jobin, Think Small: Zebrafish as a Model System of Human Pathology, *Journal*  
1340 *of Biomedicine and Biotechnology* 2012 (2012) 1-12
- 1341 [150] A.L. Menke, J.M. Spitsbergen, A.P.M. Wolterbeek, R.A. Woutersen, Normal Anatomy and  
1342 Histology of the Adult Zebrafish, *Toxicologic Pathology* 39(5) (2011) 759-775.
- 1343 [151] C.B. Kimmel, W.W. Ballard, S.R. Kimmel, B. Ullmann, T.F. Schilling, Stages of embryonic  
1344 development of the zebrafish, *Dev Dyn* 203(3) (1995) 253-310.
- 1345 [152] A. Tingaud-Sequeira, A. Knoll-Gellida, M. André, P.J. Babin, Vitellogenin expression in white  
1346 adipose tissue in female teleost fish, *Biol Reprod* 86(2) (2012) 38.
- 1347 [153] J.E. Minchin, J.F. Rawls, In vivo imaging and quantification of regional adiposity in zebrafish,  
1348 *Methods Cell Biol* 138 (2017) 3-27.
- 1349 [154] E.J. Flynn, 3rd, C.M. Trent, J.F. Rawls, Ontogeny and nutritional control of adipogenesis in  
1350 zebrafish (*Danio rerio*), *J Lipid Res* 50(8) (2009) 1641-52.
- 1351 [155] J.E.N. Minchin, J.F. Rawls, A classification system for zebrafish adipose tissues, *Dis Model Mech*  
1352 10(6) (2017) 797-809.
- 1353 [156] A. Seth, D.L. Stemple, I. Barroso, The emerging use of zebrafish to model metabolic disease,  
1354 *Disease Models & Mechanisms* 6(5) (2013) 1080-1088.
- 1355 [157] F. Maradonna, O. Carnevali, Lipid Metabolism Alteration by Endocrine Disruptors in Animal  
1356 Models: An Overview, *Front Endocrinol (Lausanne)* 9 (2018) 654.
- 1357 [158] L. Zang, L.A. Maddison, W. Chen, Zebrafish as a Model for Obesity and Diabetes, *Frontiers in cell*  
1358 *and developmental biology* 6 (2018).
- 1359 [159] E.J. Flynn, C.M. Trent, J.F. Rawls, Ontogeny and nutritional control of adipogenesis in zebrafish  
1360 (*Danio rerio*), *Journal of Lipid Research* 50(8) (2009) 1641-1652
- 1361 [160] D. Imrie, K.C. Sadler, White adipose tissue development in zebrafish is regulated by both  
1362 developmental time and fish size, *Developmental Dynamics* 239(11) (2010) 3013-3023
- 1363 [161] A. Tingaud-Sequeira, N. Ouadah, P.J. Babin, Zebrafish obesogenic test: a tool for screening  
1364 molecules that target adiposity, *J Lipid Res* 52(9) (2011) 1765-72.
- 1365 [162] N. Ouadah-Boussouf, P.J. Babin, Pharmacological evaluation of the mechanisms involved in  
1366 increased adiposity in zebrafish triggered by the environmental contaminant tributyltin, *Toxicology and*  
1367 *applied pharmacology* 294 (2016) 32-42.
- 1368 [163] E. Lutfi, P.J. Babin, J. Gutierrez, E. Capilla, I. Navarro, Caffeic acid and hydroxytyrosol have anti-  
1369 obesogenic properties in zebrafish and rainbow trout models, *PloS one* 12(6) (2017) e0178833.
- 1370 [164] L. Zang, L.A. Maddison, W. Chen, Zebrafish as a Model for Obesity and Diabetes, *Front Cell Dev*  
1371 *Biol* 6 (2018) 91.
- 1372 [165] R. Martinez, W. Tu, T. Eng, M. Allaire-Leung, B. Pina, L. Navarro-Martin, J.A. Mennigen, Acute  
1373 and long-term metabolic consequences of early developmental Bisphenol A exposure in zebrafish (*Danio*  
1374 *rerio*), *Chemosphere* 256 (2020) 127080.
- 1375 [166] J.E.N. Minchin, J.F. Rawls, In vivo Analysis of White Adipose Tissue in Zebrafish, *Methods in*  
1376 *Cell Biology*, Elsevier2011, pp. 63-86
- 1377 [167] J.E.N. Minchin, C.M. Scahill, N. Staudt, E.M. Busch-Nentwich, J.F. Rawls, Deep phenotyping in  
1378 zebrafish reveals genetic and diet-induced adiposity changes that may inform disease risk, *J Lipid Res*  
1379 59(8) (2018) 1536-1545.
- 1380 [168] A. Mentor, B. Brunström, A. Mattsson, M. Jönsson, Developmental exposure to a human relevant  
1381 mixture of endocrine disruptors alters metabolism and adipogenesis in zebrafish (*Danio rerio*),  
1382 *Chemosphere* 238 (2020) 124584.
- 1383 [169] K.E. Sant, K. Annunziato, S. Conlin, G. Teicher, P. Chen, O. Venezia, G.B. Downes, Y. Park, A.R.  
1384 Timme-Laragy, Developmental exposures to perfluorooctanesulfonic acid (PFOS) impact embryonic  
1385 nutrition, pancreatic morphology, and adiposity in the zebrafish, *Danio rerio*, *Environ Pollut* 275 (2021)  
1386 116644.
- 1387 [170] B.d.C.R. Virote, A.M.S. Moreira, J.G.d. Silva Souza, T.F.D. Castro, N. Melo, W.F. Carneiro, C.D.  
1388 Drummond, A.R.d.C.B. Vianna, L.D.S. Murgas, Obesity induction in adult zebrafish leads to negative  
1389 reproduction and offspring effects, *Reproduction* 160(6) (2020) 833-842

1390 [171] A. Tête, I. Gallais, M. Imran, L. Legoff, C. Martin-Chouly, L. Sparfel, M. Bescher, O. Sergent, N.  
1391 Podechard, D. Lagadic-Gossmann, MEHP/ethanol co-exposure favors the death of steatotic hepatocytes,  
1392 possibly through CYP4A and ADH involvement, *Food and Chemical Toxicology* 146 (2020) 111798  
1393 [172] M.M. Knuth, D. Mahapatra, D. Jima, D. Wan, B.D. Hammock, M. Law, S.W. Kullman, Vitamin D  
1394 deficiency serves as a precursor to stunted growth and central adiposity in zebrafish, *Scientific reports*  
1395 10(1) (2020) 16032.  
1396 [173] M.M. Knuth, W.L. Stutts, M.M. Ritter, K.P. Garrard, S.W. Kullman, Vitamin D deficiency  
1397 promotes accumulation of bioactive lipids and increased endocannabinoid tone in zebrafish, *J Lipid Res*  
1398 62 (2021) 100142.  
1399 [174] A. Mentor, B. Brunstrom, A. Mattsson, M. Jonsson, Developmental exposure to a human relevant  
1400 mixture of endocrine disruptors alters metabolism and adipogenesis in zebrafish (*Danio rerio*),  
1401 *Chemosphere* 238 (2020) 124584.  
1402 [175] W. Wang, X. Zhang, Z. Wang, J. Qin, W. Wang, H. Tian, S. Ru, Bisphenol S induces obesogenic  
1403 effects through deregulating lipid metabolism in zebrafish (*Danio rerio*) larvae, *Chemosphere* 199 (2018)  
1404 286-296.  
1405 [176] A.N. Buerger, J. Schmidt, A. Chase, C. Paixao, T.N. Patel, B.A. Brumback, A.S. Kane, C.J.  
1406 Martyniuk, J.H. Bisesi, Jr., Examining the responses of the zebrafish (*Danio rerio*) gastrointestinal system  
1407 to the suspected obesogen diethylhexyl phthalate, *Environ Pollut* 245 (2019) 1086-1094.  
1408 [177] A.J. Green, C. Hoyo, C.J. Mattingly, Y. Luo, J.Y. Tzeng, S.K. Murphy, D.B. Buchwalter, A.  
1409 Planchart, Cadmium exposure increases the risk of juvenile obesity: a human and zebrafish comparative  
1410 study, *Int J Obes (Lond)* 42(7) (2018) 1285-1295.  
1411 [178] A. Lyssimachou, J.G. Santos, A. Andre, J. Soares, D. Lima, L. Guimaraes, C.M. Almeida, C.  
1412 Teixeira, L.F. Castro, M.M. Santos, The Mammalian "Obesogen" Tributyltin Targets Hepatic  
1413 Triglyceride Accumulation and the Transcriptional Regulation of Lipid Metabolism in the Liver and  
1414 Brain of Zebrafish, *PloS one* 10(12) (2015) e0143911.  
1415 [179] R.K. Bhandari, Medaka as a model for studying environmentally induced epigenetic  
1416 transgenerational inheritance of phenotypes, *Environmental epigenetics* 2(1)(1) (2016) 10.  
1417 [180] Y. Tonoyama, M. Tsukada, Y. Imai, M. Sanada, S. Aota, G. Oka, S. Sugiura, N. Hori, H. Kawachi,  
1418 Y. Shimizu, N. Shimizu, Establishment of a quantitative in vivo method for estimating adipose tissue  
1419 volumes and the effects of dietary soy sauce oil on adipogenesis in medaka, *Oryzias latipes*, *PLoS One*  
1420 13(10) (2018) e0205888.  
1421 [181] X. Qiu, N. Iwasaki, K. Chen, Y. Shimasaki, Y. Oshima, Tributyltin and perfluorooctane sulfonate  
1422 play a synergistic role in promoting excess fat accumulation in Japanese medaka (*Oryzias latipes*) via in  
1423 ovo exposure, *Chemosphere* 220 (2019) 687-695.  
1424 [182] K. Chen, N. Iwasaki, X. Qiu, H. Xu, Y. Takai, K. Tashiro, Y. Shimasaki, Y. Oshima, Obesogenic  
1425 and developmental effects of TBT on the gene expression of juvenile Japanese medaka (*Oryzias latipes*),  
1426 *Aquat Toxicol* 237 (2021) 105907.  
1427 [183] B.-M. Kim, Y.J. Jo, N. Lee, N. Lee, S. Woo, J.-S. Rhee, S. Yum, Bisphenol A Induces a Distinct  
1428 Transcriptome Profile in the Male Fish of the Marine Medaka *Oryzias javanicus*, *BioChip Journal* 12  
1429 (2018) 25-37.  
1430 [184] A.T. Watson, A. Planchart, C.J. Mattingly, C. Winkler, D.M. Reif, S.W. Kullman, From the Cover:  
1431 Embryonic Exposure to TCDD Impacts Osteogenesis of the Axial Skeleton in Japanese medaka, *Oryzias*  
1432 *latipes*, *Toxicological sciences : an official journal of the Society of Toxicology* 155(2) (2017) 485-496.  
1433 [185] Yuki Takai, Takumi Takamura, Shintaro Enoki, Moeko Sato, Yoko Kato-Unoki, Xuchun Qiu,  
1434 Yohei Shimasaki, Y. Oshima, Transcriptome analysis of medaka (*Oryzias latipes*) exposed to tributyltin,  
1435 *Japanese Journal of Environmental Toxicology* 23(1) (2020) 10-21.  
1436 [186] Bo-Mi Kim, Ye Jin Jo, Nayun Lee, Nayoung Lee, Seonock Woo, Jae-Sung Rhee, S. Yum,  
1437 Bisphenol A Induces a Distinct Transcriptome Profile in the Male Fish of the Marine Medaka *Oryzias*  
1438 *javanicus*, *BioChip Journal* 12 (2018) 25-37.  
1439 [187] K. Ashrafi, Obesity and the regulation of fat metabolism, *WormBook: The Online Review of C.*  
1440 *Elegans Biology* (2007) 1-20.

1441 [188] J.L. Watts, Fat synthesis and adiposity regulation in *Caenorhabditis elegans*, Trends in  
1442 Endocrinology & Metabolism 20(2) (2009) 58-65

1443 [189] G.A. Lemieux, J. Liu, N. Mayer, R.J. Bainton, K. Ashrafi, Z. Werb, A whole-organism screen  
1444 identifies new regulators of fat storage, Nat Chem Biol 7(4) (2011) 206-13.

1445 [190] Q. Sun, Y. Yue, P. Shen, J.J. Yang, Y. Park, Cranberry Product Decreases Fat Accumulation in  
1446 *Caenorhabditis elegans*, Journal of Medicinal Food 19(4) (2016) 427-433.

1447 [191] P. Martorell, S. Llopis, N. González, F. Montón, P. Ortiz, S. Genovés, D. Ramón,  
1448 *Caenorhabditis elegans* as a Model To Study the Effectiveness and Metabolic Targets of Dietary  
1449 Supplements Used for Obesity Treatment: The Specific Case of a Conjugated Linoleic Acid Mixture  
1450 (Tonalin), Journal of Agricultural and Food Chemistry 60(44) (2012) 11071-11079

1451 [192] A. Bouyanfif, S. Jayarathne, I. Koboziev, N. Moustaid-Moussa, The Nematode *Caenorhabditis*  
1452 *elegans* as a Model Organism to Study Metabolic Effects of  $\omega$ -3 Polyunsaturated Fatty Acids in  
1453 Obesity, Advances in Nutrition 10(1) (2019) 165-178

1454 [193] B.C. Mullaney, K. Ashrafi, C. *elegans* fat storage and metabolic regulation, Biochim Biophys Acta  
1455 1791(6) (2009) 474-8.

1456 [194] R.M. McKay, J.P. McKay, L. Avery, J.M. Graff, C *elegans*: a model for exploring the genetics of  
1457 fat storage, Dev Cell 4(1) (2003) 131-42.

1458 [195] N. Mohajer, E.M. Joloya, J. Seo, T. Shioda, B. Blumberg, Epigenetic Transgenerational Inheritance  
1459 of the Effects of Obesogen Exposure, Front Endocrinol (Lausanne) 12 (2021) 787580.

1460 [196] W. Ke, J.N. Reed, C. Yang, N. Higgason, L. Rayyan, C. Wahlby, A.E. Carpenter, M. Civelek, E.J.  
1461 O'Rourke, Genes in human obesity loci are causal obesity genes in *C. elegans*, PLoS genetics 17(9)  
1462 (2021) e1009736.

1463 [197] S.W. Caito, J. Newell-Caito, M. Martell, N. Crawford, M. Aschner, Methylmercury Induces  
1464 Metabolic Alterations in *Caenorhabditis elegans*: Role for C/EBP Transcription Factor, Toxicological  
1465 sciences : an official journal of the Society of Toxicology 174(1) (2020) 112-123.

1466 [198] Z. Luo, Z. Yu, D. Yin, Obesogenic effect of erythromycin on *Caenorhabditis elegans* through over-  
1467 eating and lipid metabolism disturbances, Environ Pollut 294 (2022) 118615.

1468 [199] X. Xiao, X. Zhang, J. Bai, J. Li, C. Zhang, Y. Zhao, Y. Zhu, J. Zhang, X. Zhou, Bisphenol S  
1469 increases the obesogenic effects of a high-glucose diet through regulating lipid metabolism in  
1470 *Caenorhabditis elegans*, Food chemistry 339 (2021) 127813.

1471 [200] N. Tolwinski, Introduction: *Drosophila*—A Model System for Developmental Biology, Journal of  
1472 Developmental Biology 5(3) (2017) 9

1473 [201] M.M. Bayliak, O.B. Abrat, J.M. Storey, K.B. Storey, V.I. Lushchak, Interplay between diet-induced  
1474 obesity and oxidative stress: Comparison between *Drosophila* and mammals, Comparative Biochemistry  
1475 and Physiology Part A: Molecular & Integrative Physiology 228 (2019) 18-28

1476 [202] T.F. Bovier, D. Cavaliere, M. Colombo, G. Peluso, E. Giordano, F.A. Digilio, Methods to Test  
1477 Endocrine Disruption in *Drosophila melanogaster*, Journal of Visualized Experiments (149 ) (2019).

1478 [203] H. Cao, L. Wiemerslage, P.S.K. Marttila, M.J. Williams, H.B. Schiöth, Bis-(2-ethylhexyl) Phthalate  
1479 Increases Insulin Expression and Lipid Levels in *Drosophila melanogaster*, Basic & Clinical  
1480 Pharmacology & Toxicology 119(3) (2016) 309-316.

1481 [204] M.-Y. Chen, H.-P. Liu, J. Cheng, S.-Y. Chiang, W.-P. Liao, W.-Y. Lin, Transgenerational impact  
1482 of DEHP on body weight of *Drosophila*, Chemosphere 221 (2019) 493-499.

1483 [205] J. Zhang, Z. Yu, J. Shen, L.N. Vandenberg, D. Yin, Influences of sex, rhythm and generation on the  
1484 obesogenic potential of erythromycin to *Drosophila melanogaster*, The Science of the total environment  
1485 771 (2021) 145315.

1486 [206] S.K. Panchal, L. Brown, Rodent models for metabolic syndrome research, J Biomed Biotechnol  
1487 2011 (2011) 351982.

1488 [207] B.F. Leonardi, G. Gosmann, A.R. Zimmer, Modeling Diet-Induced Metabolic Syndrome in  
1489 Rodents, Mol Nutr Food Res 64(22) (2020) e2000249.

1490 [208] J.R. Speakman, Use of high-fat diets to study rodent obesity as a model of human obesity, Int J  
1491 Obes (Lond) 43(8) (2019) 1491-1492.

1492 [209] J.A. Taylor, B.L. Coe, T. Shioda, F.S. Vom Saal, The Crowded Uterine Horn Mouse Model for  
1493 Examining Postnatal Metabolic Consequences of Intrauterine Growth Restriction vs. Macrosomia in  
1494 Siblings, *Metabolites* 12(2) (2022).

1495 [210] J.E. Cropley, T.H. Dang, D.I. Martin, C.M. Suter, The penetrance of an epigenetic trait in mice is  
1496 progressively yet reversibly increased by selection and environment, *Proc Biol Sci* 279(1737) (2012)  
1497 2347-53.

1498 [211] J.L. Spearow, P. Doemeny, R. Sera, R. Leffler, M. Barkley, Genetic variation in susceptibility to  
1499 endocrine disruption by estrogen in mice, *Science* 285(5431) (1999) 1259-1261.

1500 [212] B. Svare, C. Kinsley, M. Mann, J. Broida, Infanticide: Accounting for genetic variation in mice.,  
1501 *Physiol. Behav.* 33 (1984) 137-152.

1502 [213] R.R. Newbold, W.N. Jefferson, E. Padilla-Banks, Prenatal exposure to bisphenol A at  
1503 environmentally relevant doses adversely affects the murine female reproductive tract later in life,  
1504 *Environmental health perspectives* 117(6) (2009) 879-85.

1505 [214] D.E. Barnard, S.M. Lewis, B.B. Teter, J.E. Thigpen, Open- and closed-formula laboratory animal  
1506 diets and their importance to research, *J Am Assoc Lab Anim Sci* 48(6) (2009) 709-13.

1507 [215] B. Kick, Want to know what to feed mice? The Jackson Labs. [https://www.jax.org/news-and-](https://www.jax.org/news-and-insights/jax-blog/2020/january/mouse-diet-guide)  
1508 [insights/jax-blog/2020/january/mouse-diet-guide](https://www.jax.org/news-and-insights/jax-blog/2020/january/mouse-diet-guide). Accessed: February 20, 2022, (2020).

1509 [216] J.J. Heindel, F.S. vom Saal, Meeting report: batch-to-batch variability in estrogenic activity in  
1510 commercial animal diets--importance and approaches for laboratory animal research, *Environmental*  
1511 *health perspectives* 116(3) (2008) 389-393.

1512 [217] J.E. Thigpen, K.D. Setchell, E. Padilla-Banks, J.K. Haseman, H.E. Saunders, G.F. Caviness, G.E.  
1513 Kissling, M.G. Grant, D.B. Forsythe, Variations in phytoestrogen content between different mill dates of  
1514 the same diet produces significant differences in the time of vaginal opening in CD-1 mice and F344 rats  
1515 but not in CD Sprague-Dawley rats, *Environmental health perspectives* 115( 12) (2007) 1717-26.

1516 [218] R. Newbold, Cellular and molecular effects of developmental exposure to diethylstilbestrol:  
1517 implications for other environmental estrogens, *Environmental health perspectives* 103(Suppl 7)  
1518 (1995) 83-87.

1519 [219] R.R. Newbold, E. Padilla-Banks, R.J. Snyder, W.N. Jefferson, Developmental exposure to  
1520 estrogenic compounds and obesity, *Birth defects research. Part A, Clinical and molecular teratology* 73(7)  
1521 (2005) 478-80.

1522 [220] D.M. Sheehan, Activity of environmentally relevant low doses of endocrine disruptors and the  
1523 bisphenol A controversy: initial results confirmed, *Proc. Soc. Exp. Biol. Med.* 224(2) (2000) 57-60.

1524 [221] S.Z. Cagen, J.M. Waechter, S.S. Dimond, W.J. Breslin, J.H. Butala, F.W. Jekat, R.L. Joiner, R.N.  
1525 Shiotsuka, G.E. Veenstra, L.R. Harris, Normal reproductive organ development in CF-1 mice following  
1526 prenatal exposure to Bisphenol A, *Tox. Sci.* 11 (1999) 15-29.

1527 [222] R.W. Tyl, C.B. Myers, M.C. Marr, B.F. Thomas, A.R. Keimowitz, D.R. Brine, M.M. Veselica, P.A.  
1528 Fail, T.Y. Chang, J.C. Seely, R.L. Joiner, J.H. Butala, S.S. Dimond, S.Z. Cagen, R.N. Shiotsuka, G.D.  
1529 Stropp, J.M. Waechter, Three-generation reproductive toxicity study of dietary bisphenol A in CD  
1530 Sprague-Dawley rats, *Toxicol. Sci.* 68(1) (2002) 121-46.

1531 [223] F.S. vom Saal, W.V. Welshons, Large effects from small exposures. II. The importance of positive  
1532 controls in low-dose research on bisphenol A, *Environmental research* 100(1) (2006) 50-76.

1533 [224] R.L. Ruhlen, J.A. Taylor, J. Mao, J. Kirkpatrick, W.V. Welshons, F.S. vom Saal, Choice of animal  
1534 feed can alter fetal steroid levels and mask developmental effects of endocrine disrupting chemicals,  
1535 *Journal of Developmental Origins of Health and Disease* 2(1) (2011) 36-48.

1536 [225] R.L. Ruhlen, K.L. Howdeshell, J. Mao, J.A. Taylor, F.H. Bronson, R.R. Newbold, W.V. Welshons,  
1537 F.S. vom Saal, Low phytoestrogen levels in feed increase fetal serum estradiol resulting in the "fetal  
1538 estrogenization syndrome" and obesity in CD-1 mice, *Environmental health perspectives* 116(3) (2008)  
1539 322-328.



1540 [226] C.R. Cederroth, M. Vinciguerra, F. Kühne, R. Madani, M. Klein, R.W. James, D.R. Doerge, T.J.  
1541 Visser, M. Foti, F. Rohner-Jeanrenaud, J.-D. Vassalli, S. Nef, A phytoestrogen-rich diet increases energy  
1542 expenditure and decreases adiposity in mice, *Environ. Health Perspect.* 115(10) (2007) 1467-1473.  
1543 [227] J.J. Heindel, B. Blumberg, M. Cave, R. Machtinger, A. Mantovani, M.A. Mendez, A. Nadal, P.  
1544 Palanza, G. Panzica, R. Sargis, L.N. Vandenberg, F.S. Vom Saal, Metabolism disrupting chemicals and  
1545 metabolic disorders, *Reprod Toxicol* 68 (2017) 3-33.  
1546 [228] K.K. Ryan, A.M. Haller, J.E. Sorrell, S.C. Woods, R.J. Jandacek, R.J. Seeley, Perinatal  
1547 exposure to bisphenol-a and the development of metabolic syndrome in CD-1 mice, *Endocrinol.*  
1548 151(6) (2010) 2603-2612.  
1549 [229] C. Wang, T. Makela, T. Hase, H. Adlercreutz, M.S. Kurzer, Lignans and flavonoids inhibit  
1550 aromatase enzyme in human preadipocytes, *J Steroid Biochem Mol Biol* 50(3-4) (1994) 205-12.  
1551 [230] M. Baker, 1,500 scientists lift the lid on reproducibility, *Nature* 533(7604) (2016) 452-4.  
1552 [231] F.S. vom Saal, C.A. Richter, R.R. Ruhlen, S.C. Nagel, B.G. Timms, W.V. Welshons, The  
1553 importance of appropriate controls, animal feed, and animal models in interpreting results from low-dose  
1554 studies of bisphenol A, *Birth Defects Res (Part A)* 73 (2005) 140-145.  
1555 [232] J.L. Spearow, Reviewer's appendix to the EPA White Paper on Species/Stock/Strain in Endocrine  
1556 Disruptor Assays. May 26, 2004.  
1557 [https://www.researchgate.net/publication/333639096\\_Reviewer's\\_Appendix\\_to\\_the\\_White\\_Paper\\_on\\_Sp](https://www.researchgate.net/publication/333639096_Reviewer's_Appendix_to_the_White_Paper_on_SpeciesStockStrain_in_Endocrine_Disruptor_Assays_Prepared_for_US_EPA_Endocrine_Disruptor_Screening_Program_Washington_DC_Work_Assignment_No_4-5_Task_No16)  
1558 [eciesStockStrain\\_in\\_Endocrine\\_Disruptor\\_Assays\\_Prepared\\_for\\_US\\_EPA\\_Endocrine\\_Disruptor\\_Screen](https://www.researchgate.net/publication/333639096_Reviewer's_Appendix_to_the_White_Paper_on_SpeciesStockStrain_in_Endocrine_Disruptor_Assays_Prepared_for_US_EPA_Endocrine_Disruptor_Screening_Program_Washington_DC_Work_Assignment_No_4-5_Task_No16)  
1559 [ing\\_Program\\_Washington\\_DC\\_Work\\_Assignment\\_No\\_4-5\\_Task\\_No16](https://www.researchgate.net/publication/333639096_Reviewer's_Appendix_to_the_White_Paper_on_SpeciesStockStrain_in_Endocrine_Disruptor_Assays_Prepared_for_US_EPA_Endocrine_Disruptor_Screening_Program_Washington_DC_Work_Assignment_No_4-5_Task_No16) Accessed: June 6, 2021, (2004).  
1560 [233] P.A. Hunt, K.E. Koehler, M. Susiarjo, C.A. Hodges, A. Ilagan, R.C. Voigt, S. Thomas, B.F.  
1561 Thomas, T.J. Hassold, Bisphenol a exposure causes meiotic aneuploidy in the female mouse, *Curr Biol*  
1562 13(7) (2003) 546-53.  
1563 [234] K.E. Koehler, R.C. Voigt, S. Thomas, B. Lamb, C. Urban, T.J. Hassold, P.A. Hunt, When disaster  
1564 strikes: Rethinking caging materials, *Lab animal* 32 (2003) 32-35.  
1565 [235] K.L. Howdeshell, P.H. Peterman, B.M. Judy, J.A. Taylor, C.E. Orazio, R.L. Ruhlen, F.S. Vom  
1566 Saal, W.V. Welshons, Bisphenol A is released from used polycarbonate animal cages into water at room  
1567 temperature, *Environ Health Perspect* 111(9) (2003) 1180-1187.  
1568 [236] F. Lizcano, F. Arroyave, Control of Adipose Cell Browning and Its Therapeutic Potential,  
1569 *Metabolites* 10(11) (2020).  
1570 [237] S. Kajimura, B.M. Spiegelman, P. Seale, Brown and Beige Fat: Physiological Roles beyond Heat  
1571 Generation, *Cell Metab* 22(4) (2015) 546-59.  
1572 [238] A. Galmozzi, S.B. Sonne, S. Altshuler-Keylin, Y. Hasegawa, K. Shinoda, I.H.N. Luijten, J.W.  
1573 Chang, L.Z. Sharp, B.F. Cravatt, E. Saez, S. Kajimura, ThermoMouse: an in vivo model to identify  
1574 modulators of UCP1 expression in brown adipose tissue, *Cell Rep* 9(5) (2014) 1584-1593.  
1575 [239] L. Kazak, E.T. Chouchani, M.P. Jedrychowski, B.K. Erickson, K. Shinoda, P. Cohen, R.  
1576 Vetrivelan, G.Z. Lu, D. Laznik-Bogoslavski, S.C. Hasenfuss, S. Kajimura, S.P. Gygi, B.M. Spiegelman,  
1577 A creatine-driven substrate cycle enhances energy expenditure and thermogenesis in beige fat, *Cell*  
1578 163(3) (2015) 643-55.  
1579 [240] I.G. Shabalina, T.V. Kramarova, J. Nedergaard, B. Cannon, Carboxyatractyloside effects on brown-  
1580 fat mitochondria imply that the adenine nucleotide translocator isoforms ANT1 and ANT2 may be  
1581 responsible for basal and fatty-acid-induced uncoupling respectively, *Biochem J* 399(3) (2006) 405-14.  
1582 [241] J.Z. Long, K.J. Svensson, L.A. Bateman, H. Lin, T. Kamenecka, I.A. Lokurkar, J. Lou, R.R. Rao,  
1583 M.R. Chang, M.P. Jedrychowski, J.A. Paulo, S.P. Gygi, P.R. Griffin, D.K. Nomura, B.M. Spiegelman,  
1584 The Secreted Enzyme PM20D1 Regulates Lipidated Amino Acid Uncouplers of Mitochondria, *Cell*  
1585 166(2) (2016) 424-435.  
1586 [242] W.Y. Park, J. Park, S. Lee, G. Song, I.K. Nam, K.S. Ahn, S.K. Choe, J.Y. Um, PEX13 is required  
1587 for thermogenesis of white adipose tissue in cold-exposed mice, *Biochim Biophys Acta Mol Cell Biol*  
1588 *Lipids* 1867(1) (2022) 159046.

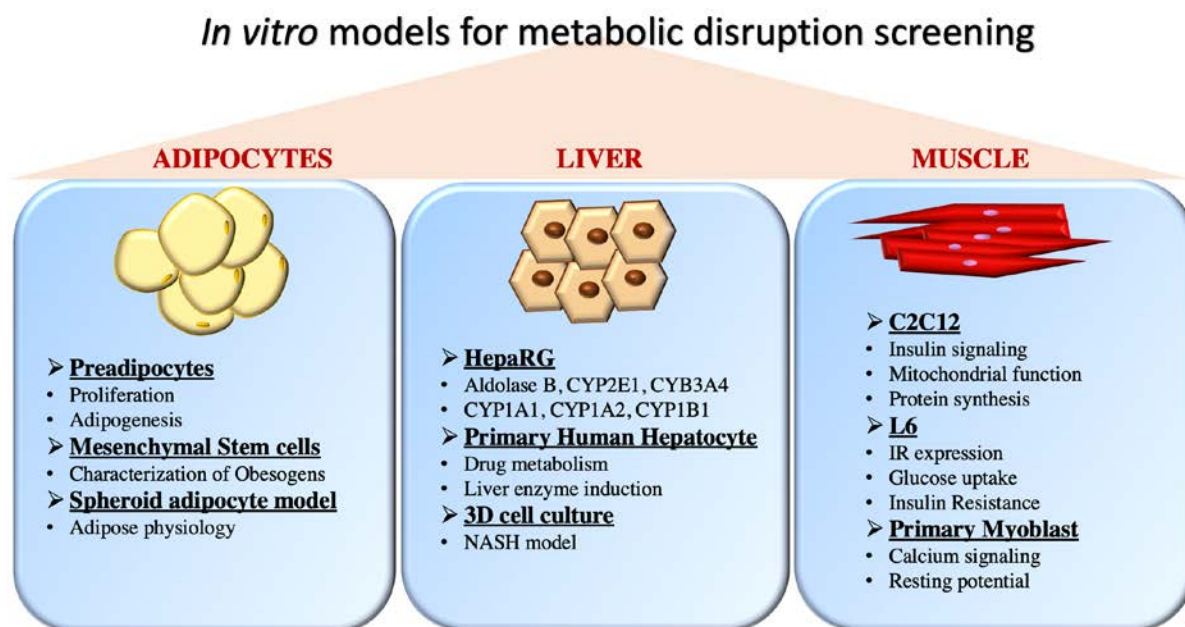
1589 [243] M.M. Snyder, F. Yue, L. Zhang, R. Shang, J. Qiu, J. Chen, K.H. Kim, Y. Peng, S.N. Oprescu, S.S.  
1590 Donkin, P. Bi, S. Kuang, LETMD1 is required for mitochondrial structure and thermogenic function of  
1591 brown adipocytes, *FASEB J* 35(11) (2021) e21965.  
1592 [244] D.I. Kim, J. Liao, M.P. Emont, M.J. Park, H. Jun, S.K. Ramakrishnan, J.D. Lin, Y.M. Shah, M.B.  
1593 Omary, J. Wu, An OLTAM system for analysis of brown/beige fat thermogenic activity, *Int J Obes*  
1594 (Lond) 42(4) (2018) 939-945.  
1595 [245] M.A. Antonacci, C. McHugh, M. Kelley, A. McCallister, S. Degan, R.T. Branca, Direct detection  
1596 of brown adipose tissue thermogenesis in UCP1<sup>-/-</sup> mice by hyperpolarized (129)Xe MR thermometry, *Sci*  
1597 *Rep* 9(1) (2019) 14865.  
1598 [246] R.T. Branca, A. McCallister, H. Yuan, A. Aghajanian, J.E. Faber, N. Weimer, R. Buchanan, C.S.  
1599 Floyd, M. Antonacci, L. Zhang, A. Burant, Accurate quantification of brown adipose tissue mass by  
1600 xenon-enhanced computed tomography, *Proc Natl Acad Sci U S A* 115(1) (2018) 174-179.  
1601 [247] R. Kriszt, S. Arai, H. Itoh, M.H. Lee, A.G. Goralczyk, X.M. Ang, A.M. Cypess, A.P. White, F.  
1602 Shamsi, R. Xue, J.Y. Lee, S.C. Lee, Y. Hou, T. Kitaguchi, T. Sudhakaran, S. Ishiwata, E.B. Lane, Y.T.  
1603 Chang, Y.H. Tseng, M. Suzuki, M. Raghunath, Optical visualisation of thermogenesis in stimulated  
1604 single-cell brown adipocytes, *Sci Rep* 7(1) (2017) 1383.  
1605 [248] S. Kajimura, Promoting brown and beige adipocyte biogenesis through the PRDM16 pathway, *Int J*  
1606 *Obes Suppl* 5(Suppl 1) (2015) S11-4.  
1607 [249] T. Knudsen, M. Martin, K. Chandler, N. Kleinstreuer, R. Judson, N. Sipes, Predictive models and  
1608 computational toxicology, *Methods in molecular biology* (Clifton, N.J.) 947 (2013) 343-74.  
1609 [250] S.K. Bopp, A. Kienzler, A.N. Richarz, S.C. van der Linden, A. Pains, N. Parissis, A.P. Worth,  
1610 Regulatory assessment and risk management of chemical mixtures: challenges and ways forward, *Crit*  
1611 *Rev Toxicol* 49(2) (2019) 174-189.  
1612 [251] Q. Wu, X. Coumoul, P. Grandjean, R. Barouki, K. Audouze, Endocrine disrupting chemicals and  
1613 COVID-19 relationships: a computational systems biology approach, *medRxiv* (2020).  
1614 [252] K. Audouze, S. Brunak, P. Grandjean, A computational approach to chemical etiologies of diabetes,  
1615 *Scientific reports* 3 (2013) 2712.  
1616 [253] G.T. Ankley, R.S. Bennett, R.J. Erickson, D.J. Hoff, M.W. Hornung, R.D. Johnson, D.R. Mount,  
1617 J.W. Nichols, C.L. Russom, P.K. Schmieder, J.A. Serrano, J.E. Tietge, D.L. Villeneuve, Adverse  
1618 outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment,  
1619 *Environmental toxicology and chemistry / SETAC* 29(3) (2010) 730-41.  
1620 [254] T. Svingen, D.L. Villeneuve, D. Knapen, E.M. Panagiotou, M.K. Draskau, P. Damdimopoulou,  
1621 J.M. O'Brien, A Pragmatic Approach to Adverse Outcome Pathway Development and Evaluation,  
1622 *Toxicological sciences : an official journal of the Society of Toxicology* 184(2) (2021) 183-190.  
1623 [255] F.S. Vom Saal, L.N. Vandenberg, Update on the Health Effects of Bisphenol A: Overwhelming  
1624 Evidence of Harm, *Endocrinology* 162(3) (2021).  
1625 [256] N.O. Oki, S.W. Edwards, An integrative data mining approach to identifying adverse outcome  
1626 pathway signatures, *Toxicology* 350-352 (2016) 49-61.  
1627 [257] F. Jornod, T. Jaylet, L. Blaha, D. Sarigiannis, L. Tamisier, K. Audouze, AOP-helpFinder  
1628 webserver: a tool for comprehensive analysis of the literature to support adverse outcome pathways  
1629 development, *Bioinformatics* (2021).  
1630 [258] J.C. Carvaillo, R. Barouki, X. Coumoul, K. Audouze, Linking Bisphenol S to Adverse Outcome  
1631 Pathways Using a Combined Text Mining and Systems Biology Approach, *Environ Health Perspect*  
1632 127(4) (2019) 47005.  
1633 [259] M. Rugard, X. Coumoul, J.C. Carvaillo, R. Barouki, K. Audouze, Deciphering Adverse Outcome  
1634 Pathway Network Linked to Bisphenol F Using Text Mining and Systems Toxicology Approaches,  
1635 *Toxicological sciences : an official journal of the Society of Toxicology* 173(1) (2020) 32-40.  
1636 [260] T.T. Schug, R. Abagyan, B. Blumberg, T.J. Collins, D. Crews, P.L. DeFur, S.M. Dickerson, T.M.  
1637 Edwards, A.C. Gore, L.J. Guillette, T. Hayes, J.J. Heindel, A. Moores, H.B. Patisaul, T.L. Tal, K.A.  
1638 Thayer, L.N. Vandenberg, J. Warner, C.S. Watson, F.S. vom Saal, R.T. Zoeller, K.P. O'Brien, J.P. Myers,

1639 Designing Endocrine Disruption Out of the Next Generation of Chemicals, *Green chemistry : an*  
1640 *international journal and green chemistry resource : GC* 15(1) (2013) 181-198.  
1641 [261] K. Audouze, D. Sarigiannis, P. Alonso-Magdalena, C. Brochot, M. Casas, M. Vrijheid, P.J. Babin,  
1642 S. Karakitsios, X. Coumoul, R. Barouki, Integrative Strategy of Testing Systems for Identification of  
1643 Endocrine Disruptors Inducing Metabolic Disorders-An Introduction to the OBERON Project,  
1644 *International journal of molecular sciences* 21(8) (2020) 2988.  
1645 [262] K.A. Thayer, J.J. Heindel, J.R. Bucher, M.A. Gallo, Role of environmental chemicals in diabetes  
1646 and obesity: a National Toxicology Program workshop review, *Environmental health perspectives* 120(6)  
1647 (2012) 779-89.  
1648 [263] E. Zgheib, M.J. Kim, F. Jornod, K. Bernal, C. Tomkiewicz, S. Bortoli, X. Coumoul, R. Barouki, K.  
1649 De Jesus, E. Grignard, P. Hubert, E.S. Katsanou, F. Busquet, K. Audouze, Identification of non-validated  
1650 endocrine disrupting chemical characterization methods by screening of the literature using artificial  
1651 intelligence and by database exploration, *Environment international* 154 (2021) 106574.  
1652 [264] M.E. Street, K. Audouze, J. Legler, H. Sone, P. Palanza, Endocrine Disrupting Chemicals: Current  
1653 Understanding, New Testing Strategies and Future Research Needs, *Int J Mol Sci* 22(2) (2021).  
1654 [265] K. Audouze, D. Sarigiannis, P. Alonso-Magdalena, C. Brochot, M. Casas, M. Vrijheid, P.J. Babin,  
1655 S. Karakitsios, X. Coumoul, R. Barouki, Integrative Strategy of Testing Systems for Identification of  
1656 Endocrine Disruptors Inducing Metabolic Disorders-An Introduction to the OBERON Project, *Int J Mol*  
1657 *Sci* 21(8) (2020).  
1658 [266] J. Legler, D. Zalko, F. Jourdan, M. Jacobs, B. Fromenty, P. Balaguer, W. Bourguet, V. Munic Kos,  
1659 A. Nadal, C. Beausoleil, S. Cristobal, S. Remy, S. Ermler, L. Margiotta-Casaluci, J.L. Griffin, B.  
1660 Blumberg, C. Chesne, S. Hoffmann, P.L. Andersson, J.H. Kamstra, The GOLIATH Project: Towards an  
1661 Internationally Harmonised Approach for Testing Metabolism Disrupting Compounds, *Int J Mol Sci*  
1662 21(10) (2020).  
1663 [267] J. Kublbeck, T. Vuorio, J. Niskanen, V. Fortino, A. Braeuning, K. Abass, A. Rautio, J. Hakkola, P.  
1664 Honkakoski, A.L. Levonen, The EDCMET Project: Metabolic Effects of Endocrine Disruptors, *Int J Mol*  
1665 *Sci* 21(8) (2020).  
1666 [268] M.A. La Merrill, L.N. Vandenberg, M.T. Smith, W. Goodson, P. Browne, H.B. Patisaul, K.Z.  
1667 Guyton, A. Kortenkamp, V.J. Cogliano, T.J. Woodruff, L. Rieswijk, H. Sone, K.S. Korach, A.C. Gore, L.  
1668 Zeise, R.T. Zoeller, Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for  
1669 hazard identification, *Nat Rev Endocrinol* 16(1) (2020) 45-57.  
1670 [269] I. Rusyn, X. Arzuaga, R.C. Cattley, J.C. Corton, S.S. Ferguson, P. Godoy, K.Z. Guyton, N.  
1671 Kaplowitz, S.R. Khetani, R. Roberts, R.A. Roth, M.T. Smith, Key Characteristics of Human  
1672 Hepatotoxicants as a Basis for Identification and Characterization of the Causes of Liver Toxicity,  
1673 *Hepatology* n/a(n/a) (2021).  
1674 [270] J.Y. Kim, S.J. Kim, M.A. Bae, J.R. Kim, K.H. Cho, Cadmium exposure exacerbates severe  
1675 hyperlipidemia and fatty liver changes in zebrafish via impairment of high-density lipoproteins  
1676 functionality, *Toxicology in vitro : an international journal published in association with BIBRA* 47  
1677 (2018) 249-258.  
1678 [271] J.L. Lyche, R. Nourizadeh-Lillabadi, C. Almaas, B. Stavik, V. Berg, J.U. Skare, P. Alestrom, E.  
1679 Ropstad, Natural mixtures of persistent organic pollutants (POP) increase weight gain, advance puberty,  
1680 and induce changes in gene expression associated with steroid hormones and obesity in female zebrafish,  
1681 *Journal of toxicology and environmental health. Part A* 73(15) (2010) 1032-57.  
1682 [272] S. Santangeli, V. Notarstefano, F. Maradonna, E. Giorgini, G. Gioacchini, I. Forner-Piquer, H.R.  
1683 Habibi, O. Carnevali, Effects of diethylene glycol dibenzoate and Bisphenol A on the lipid metabolism of  
1684 *Danio rerio*, *The Science of the total environment* 636 (2018) 641-655.  
1685 [273] A.N. Buerger, D.T. Dillon, J. Schmidt, T. Yang, J. Zubcevic, C.J. Martyniuk, J.H. Bisesi, Jr.,  
1686 Gastrointestinal dysbiosis following diethylhexyl phthalate exposure in zebrafish (*Danio rerio*): Altered  
1687 microbial diversity, functionality, and network connectivity, *Environ Pollut* 265(Pt B) (2020) 114496.  
1688 [274] S. Bucher, A. Tete, N. Podechard, M. Liamin, D. Le Guillou, M. Chevanne, C. Coulouarn, M.  
1689 Imran, I. Gallais, M. Fernier, Q. Hamdaoui, M.A. Robin, O. Sergent, B. Fromenty, D. Lagadic-

1690 Gossmann, Co-exposure to benzo[a]pyrene and ethanol induces a pathological progression of liver  
1691 steatosis in vitro and in vivo, *Scientific reports* 8(1) (2018) 5963.  
1692 [275] M. Imran, O. Sergent, A. Tete, I. Gallais, M. Chevanne, D. Lagadic-Gossmann, N. Podechard,  
1693 Membrane Remodeling as a Key Player of the Hepatotoxicity Induced by Co-Exposure to  
1694 Benzo[a]pyrene and Ethanol of Obese Zebrafish Larvae, *Biomolecules* 8(2) (2018).  
1695 [276] L. Sun, Y. Ling, J. Jiang, D. Wang, J. Wang, J. Li, X. Wang, H. Wang, Differential mechanisms  
1696 regarding triclosan vs. bisphenol A and fluorene-9-bisphenol induced zebrafish lipid-metabolism  
1697 disorders by RNA-Seq, *Chemosphere* 251 (2020) 126318.  
1698 [277] A. Martella, C. Silvestri, F. Maradonna, G. Gioacchini, M. Allara, G. Radaelli, D.R. Overby, V. Di  
1699 Marzo, O. Carnevali, Bisphenol A Induces Fatty Liver by an Endocannabinoid-Mediated Positive  
1700 Feedback Loop, *Endocrinology* 157(5) (2016) 1751-63.  
1701 [278] L. Renaud, W.A.D. Silveira, E.S. Hazard, J. Simpson, S. Falcinelli, D. Chung, O. Carnevali, G.  
1702 Hardiman, The Plasticizer Bisphenol A Perturbs the Hepatic Epigenome: A Systems Level Analysis of  
1703 the miRNome, *Genes* 8(10) (2017).  
1704 [279] J. Qin, S. Ru, W. Wang, L. Hao, Y. Ru, J. Wang, X. Zhang, Long-term bisphenol S exposure  
1705 aggravates non-alcoholic fatty liver by regulating lipid metabolism and inducing endoplasmic reticulum  
1706 stress response with activation of unfolded protein response in male zebrafish, *Environ Pollut* 263(Pt B)  
1707 (2020) 114535.  
1708 [280] W. Wang, X. Zhang, J. Qin, P. Wei, Y. Jia, J. Wang, S. Ru, Long-term bisphenol S exposure  
1709 induces fat accumulation in liver of adult male zebrafish (*Danio rerio*) and slows yolk lipid consumption  
1710 in F1 offspring, *Chemosphere* 221 (2019) 500-510.  
1711 [281] A. Tete, I. Gallais, M. Imran, L. Legoff, C. Martin-Chouly, L. Sparfel, M. Bescher, O. Sergent, N.  
1712 Podechard, D. Lagadic-Gossmann, MEHP/ethanol co-exposure favors the death of steatotic hepatocytes,  
1713 possibly through CYP4A and ADH involvement, *Food and chemical toxicology : an international journal*  
1714 *published for the British Industrial Biological Research Association* 146 (2020) 111798.  
1715 [282] M. Huff, W.A. da Silveira, O. Carnevali, L. Renaud, G. Hardiman, Systems Analysis of the Liver  
1716 Transcriptome in Adult Male Zebrafish Exposed to the Plasticizer (2-Ethylhexyl) Phthalate (DEHP),  
1717 *Scientific reports* 8(1) (2018) 2118.  
1718 [283] W. Qiu, S. Liu, F. Yang, P. Dong, M. Yang, M. Wong, C. Zheng, Metabolism disruption analysis  
1719 of zebrafish larvae in response to BPA and BPA analogs based on RNA-Seq technique, *Ecotoxicology*  
1720 *and environmental safety* 174 (2019) 181-188.  
1721 [284] H.M. Jacobs, K.E. Sant, A. Basnet, L.M. Williams, J.B. Moss, A.R. Timme-Laragy, Embryonic  
1722 exposure to Mono(2-ethylhexyl) phthalate (MEHP) disrupts pancreatic organogenesis in zebrafish (*Danio*  
1723 *rerio*), *Chemosphere* 195 (2018) 498-507.  
1724 [285] X. Zhou, J. Li, X. Zhang, C. Zhang, J. Bai, Y. Zhao, Y. Zhu, J. Zhang, X. Xiao, Bisphenol S  
1725 promotes fat storage in multiple generations of *Caenorhabditis elegans* in a *daf-16/nhr-49* dependent  
1726 manner, *Comparative biochemistry and physiology. Toxicology & pharmacology : CBP* 250 (2021)  
1727 109175.  
1728 [286] Z. Li, Z. Yu, P. Gao, D. Yin, Multigenerational effects of perfluorooctanoic acid on lipid  
1729 metabolism of *Caenorhabditis elegans* and its potential mechanism, *The Science of the total environment*  
1730 703 (2020) 134762.  
1731 [287] M.Y. Chen, H.P. Liu, J. Cheng, S.Y. Chiang, W.P. Liao, W.Y. Lin, Transgenerational impact of  
1732 DEHP on body weight of *Drosophila*, *Chemosphere* 221 (2019) 493-499.  
1733 [288] J. Wang, Y. Li, Y. Liu, H. Zhang, J. Dai, Disturbance of perfluorooctanoic acid on development  
1734 and behavior in *Drosophila* larvae, *Environmental toxicology and chemistry / SETAC* 29(9) (2010) 2117-  
1735 22.  
1736 [289] J.H. Kim, B. Barbagallo, K. Annunziato, R. Farias-Pereira, J.J. Doherty, J. Lee, J. Zina, C. Tindal,  
1737 C. McVey, R. Aresco, M. Johnstone, K.E. Sant, A. Timme-Laragy, Y. Park, J.M. Clark, Maternal  
1738 preconception PFOS exposure of *Drosophila melanogaster* alters reproductive capacity, development,  
1739 morphology and nutrient regulation, *Food and chemical toxicology : an international journal published*  
1740 *for the British Industrial Biological Research Association* 151 (2021) 112153.

1741

1742








1744

1745 **Figure 1:** *In vitro* models used for testing the effect of metabolic disrupting chemicals on various

1746 pathways. Common uses of the various cell models are described.

## In vivo models for metabolic disruption screening

Models	Advantages	Disadvantages
<b>Zebrafish</b> 	<ul style="list-style-type: none"> <li>• Rapid development, ease of breeding, transparency</li> <li>• Metabolic organs/tissues are physiologically and anatomically similar to humans</li> <li>• High-resolution fluorescent imaging of total body adipose</li> <li>• Ease of molecular manipulation, wealth of transgenic models</li> </ul>	<ul style="list-style-type: none"> <li>• Moderate flexibility</li> <li>• Moderate translational value</li> </ul>
<b>Medaka</b> 	<ul style="list-style-type: none"> <li>• Genetic sex determination like humans</li> <li>• Rapid development, ease of breeding, transparency</li> <li>• Metabolic organs/tissues are physiologically and anatomically similar to humans</li> <li>• Ease of molecular manipulation, small genome size, high diversity</li> </ul>	<ul style="list-style-type: none"> <li>• Moderate flexibility</li> <li>• Moderate translational value</li> <li>• Less characterization of adipose relative to zebrafish</li> </ul>
<b><i>C. elegans</i></b> 	<ul style="list-style-type: none"> <li>• Compounds that modulate fat storage and obesity can be identified</li> <li>• Food intake and energy expenditure can be measured easily</li> <li>• Less regulations governing invertebrate animal use</li> </ul>	<ul style="list-style-type: none"> <li>• Lower conservation of biological pathways with mammals</li> <li>• Lack of specific organs and circulatory systems</li> </ul>
<b><i>D. melanogaster</i></b> 	<ul style="list-style-type: none"> <li>• Small size, short generation time, inexpensive and easy breeding</li> <li>• Several discrete organs perform the same as humans</li> <li>• Less regulations governing invertebrate animal use</li> </ul>	<ul style="list-style-type: none"> <li>• Anatomically different from mammals</li> <li>• Lower conservation of many relevant biological pathways with mammals</li> </ul>
<b>Rodents</b> 	<ul style="list-style-type: none"> <li>• Well described model with clear translation to human outcomes</li> <li>• Periconception, pregnancy, parental and offspring, short- and long-term, multi- and trans-generational outcomes can be assessed</li> <li>• Diverse housing materials readily available</li> <li>• Well-characterized &amp; customizable feed options readily available</li> <li>• Inbred and outbred models available to dissect role of genes, environment, and their interactions</li> </ul>	<ul style="list-style-type: none"> <li>• Time consuming and expensive compared to above alternatives, but less so with larger animal models (e.g. porcine, bovine, ovine, and non-human primates).</li> <li>• Ethical issues; regulatory push to reduce use of mammalian vertebrate animal models</li> </ul>

1747

1748 **Figure 2:** Advantages and disadvantages of *in vivo* models for metabolic disrupting chemical evaluation.  
 1749 Common or emerging model organisms used in metabolic health research are discussed and various  
 1750 characteristics are described.

1751

1752 **Table 1: Obesogenic chemical testing in emerging *in vivo* models (zebrafish, medaka, roundworm,**  
 1753 **fruit fly)**

1754

<b>Species</b>	<b>Mode of action</b>	<b>Representative References</b>
Danio rerio	<b>Obesity phenotype</b> Increased weight, adiposity, and/or lipid accumulation	Cadmium: [177], [270] DDT mixture: [271] Nonylphenol and polyethoxylates: [79] Bisphenols: [272], [27], [165] Phthalates: [176], [273], [174] PFOS: [169]
	<b>NAFLD phenotype</b> Steatosis, fatty liver changes	Cadmium: [270] Benzo(a)pyrene: [274], [275] Bisphenols: [276], [277], [278], [279], [280] Phthalates: [281], [282]
	<b>Metabolism changes</b> Metabolomics, lipids, fatty acids, diabetic phenotype, etc.	Bisphenols: [283], [278], [165] Phthalates: [176], [284], [174] PFOS: [169]
Oryzias latipes	<b>Obesity phenotype</b> Increased weight, adiposity, and/or lipid accumulation	TBT: [182] TBT/PFOS: [181]
	<b>NAFLD phenotype</b> Steatosis, fatty liver changes	
	<b>Metabolism changes</b> Metabolomics, lipids, fatty acids, diabetic phenotype, etc.	TBT: [182] Bisphenols: [186]
C. elegans	<b>Obesity phenotype</b> Increased weight, adiposity, and/or lipid accumulation	Bisphenols: [199], [285] Erythromycin: [198] PFOA: [286]
	<b>NAFLD phenotype</b> Steatosis, fatty liver	
	<b>Metabolism changes</b> Metabolomics, lipids, fatty acids, diabetic phenotype, etc.	Bisphenols: [199] Erythromycin: [198] Methylmercury: [197] PFOA: [286]
Drosophila melanogaster	<b>Obesity phenotype</b> Increased weight, adiposity, and/or lipid accumulation	DEHP: [287]
	<b>NAFLD phenotype</b> Steatosis, fatty liver changes	
	<b>Metabolism changes</b> Metabolomics, lipids, fatty acids, diabetic phenotype, etc.	PFOA: [288] PFOS: [289]

1755

1756 Summary table of obesogenic activity testing in the zebrafish, medaka, roundworm, and fruit fly models.

1757 Representative obesogenic chemical testing (non-exhaustive) is included to detail the diversity of

1758 contaminants examined.

1759