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- Effects of Developmental Lead and Phthalate Exposures on DNA Methylation in Adult Mouse

2 Blood, Brain, and Liver Identifies Tissue- and Sex-Specific Changes with Implications for Gene

1 Imprinting

8 Rachel K. Morgan^{1,} Rachel K. Morgan^{1,†}, Kai Wang^{2,†}, Laurie K. Svoboda¹, Christine A. Rygiel¹, Claudia Lalancette³,
Rachel K. Morgan^{1,†}, Kai Wang^{2,†}, Laurie K. Svoboda¹, Christine A. Rygiel¹, Claudia Lalancette³,
Raymond
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- **Rachel K. Morgan1, † , Kai Wang2, † , Laurie K. Svoboda¹ , Christine A. Rygiel¹ , Claudia Lalancette3**
- **Raymond Cavalcante3 , Marisa S. Bartolomei⁴ , Rexxi Prasasya4 , Kari Neier¹**
- **Perera¹ , Tamara R Jones¹ , Justin A. Colacino1,5, Maureen A. Sartor2,6, Dana C. Dolinoy1,5 ***
- 3 **Imprinting**
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5 **Rachel K. N**
6 **Raymond C**
7 **Perera¹, Tal**
8 ¹ Department 56789 Rachel K. Morgan^{1, T}, Kai Wang^{2, T}, Laurie K. Svoboda¹, Christine A. Rygiel¹, Claudia Lalancette³,

Raymond Cavalcante³, Marisa S. Bartolomei⁴, Rexxi Prasasya⁴, Kari Neier¹, Bambarendage P.U.

Perera¹, **Raymond Cavalcante³, Marisa S. Bartolomei⁴, Rexxi Prasasya⁴, Kari Neier¹, Bambarendage P.U.

Perera¹, Tamara R Jones¹, Justin A. Colacino^{1,5}, Maureen A. Sartor^{2,6}, Dana C. Dolinoy^{1,5*}

¹Department of E** 89012 ¹Department of Environmental Health Sciences, School of Public Health, University of Michigan, Ann ² Department of Environmental Health Sciences, School of Public Health, University of Michigan, Ann

² Department of Computational Medicine and Bioinformatics, School of Medicine, University of

² Department of Compu
- 2
-
- ³Epigenomics Core, School of Medicine, University of Michigan, Ann Arbor, MI 48109, USA
- 9 Arbor, MI 48109, USA

0 ² Department of Comput

1 Michigan, Ann Arbor, N

2 ³ Epigenomics Core, Sch

4 ⁴ Department of Cell and

4 Beralman School of Ma ² Department of Computational Medicine and Bioinformatics, School of Medicine, University of Michigan, Ann Arbor, MI 48109, USA
³ Epigenomics Core, School of Medicine, University of Michigan, Ann Arbor, MI 48109, USA
 ⁴Department of Cell and Developmental Biology, Center of Excellence in Environmental Toxicology,
-
- Michigan, Ann Arbor, MI 48109, USA

³Epigenomics Core, School of Medicine

⁴Department of Cell and Developmenta

⁴Perelman School of Medicine, Universi

⁵Department of Nutritional Sciences, Sc ³Epigenomics Core, School of Medicine, University of Michigan, Ann Arbor, MI 48109, USA
⁴Department of Cell and Developmental Biology, Center of Excellence in Environmental Toxic
Perelman School of Medicine, University ⁴Department of Cell and Developmental Biology, Center of Excellence in Environmental Toxicology,

14 Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

⁵Department of Nutritional Scie 14 Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

⁵Department of Nutritional Sciences, School of Public Health, University of Michigan, An

¹⁶ ⁶Department of Biostatistics, Scho ⁵Department of Nutritional Sciences, School of Public Health, University of Michigan, Ann Arbor, MI
- The ^SDepartment of Nutritional Sciences, School of Public Health, University of Michigan, Ann Arbor, MI

16 48109, USA

⁶Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI 48109 16 48109, USA

17 ⁶Department

18 USA

19 †These autho

21 *Correspond ⁶Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI 48109, ^oDepartment of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI 48109,
18 USA
20 †These authors contributed equally
^{*}Correspondence:
22 Dana C. Dolinoy
23 ddolinoy@umich.edu
-
-

-
- 18 USA
19
20 †Thes
21 ***Cor**
22 Dana
23 <u>ddoli</u>
24 6671 20
22
23
24
25
-
-
- 20 †These authors contributed equally

21 *Correspondence:

22 Dana C. Dolinoy

23 ddolinoy@umich.edu

24 6671 SPH I

25 1415 Washington Heights

26 Ann Arbor, MI 48109-2029 21 ***Correspondence:**

22 Dana C. Dolinoy

23 <u>ddolinoy@umich.ed</u>

24 6671 SPH I

25 1415 Washington H

26 Ann Arbor, MI 4810

27 **Conflicts of Interes** 22 Dana C. Dolinoy

23 <u>ddolinoy@umich</u>

24 6671 SPH I

25 1415 Washington

26 Ann Arbor, MI 48

27 **Conflicts of Inter**

29 *The authors decle* 23 ddolinoy@umich.edu

24 6671 SPH I

25 1415 Washington Hei

26 Ann Arbor, MI 48109

27 **Conflicts of Interest**

29 *The authors declare t.*

30 24 6671 SPH I
25 1415 Washin
26 Ann Arbor, 27
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29 *The authors*
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30 29 *The authors declare they have nothing to disclose.*
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- 31 **Abstract**
32 **Backgrou**
34 offspring.
35 modificati
35 **Objective**
37 **human-rel in cerebra** - 3
3 3 4 5 6 7 8 9 0
3 3 3 3 3 9 0 **Background:** Maternal exposure to environmental chemicals can cause adverse health effects in

34 offspring. Mounting evidence supports that these effects are influenced, at least in part, by epigen

35 objective: We exam offspring. Mounting evidence supports that these effects are influenced, at least in part, by epigenetic
 35 objective: We examined tissue- and sex-specific changes in DNA methylation (DNAm) associated w

human-relevant
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- 35 modifications.

36 **Objective:** We

37 human-relevan

in cerebral cort

39 **Methods:** Fem

40 **DEHP** (5 mg/k

41 bisulfite sequen

42 liver at 5 montl

42 (NARe) Anno
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- **36 Objective:** We examined tissue- and sex-specific changes in DNA methylation (DNAm) associated with

137 human-relevant lead (Pb) and di(2-ethylhexyl) phthalate (DEHP) exposure during perinatal development

138 in cer 37 human-relevant lead (Pb) and di(2-ethylhexyl) phthalate (DEHP) exposure during perinatal development
38 in cerebral cortex, blood, and liver.
39 **Methods:** Female mice were exposed to human relevant doses of either Pb (38 in cerebral cortex, blood, and liver.
39 **Methods:** Female mice were expose
40 **DEHP** (5 mg/kg-day) via chow for t
41 bisulfite sequencing (WGBS) was u
42 liver at 5 months of age. Metilene at
44 **DMRs**). Annotatr and C **39 Methods:** Female mice were exposed to human relevant doses of either Pb (32ppm) via drinking water or

40 DEHP (5 mg/kg-day) via chow for two weeks prior to mating through offspring weaning. Whole genome

41 bisulfite 40 DEHP (5 mg/kg-day) via chow for two weeks prior to mating through offspring weaning. Whole genome
41 bisulfite sequencing (WGBS) was utilized to examine DNAm changes in offspring cortex, blood, and
42 liver at 5 months bisulfite sequencing (WGBS) was utilized to examine DNAm changes in offspring cortex, blood, and

42 liver at 5 months of age. Metilene and methylSig were used to identify differentially methylated region

43 (DMRs). Annot liver at 5 months of age. Metilene and methylSig were used to identify differentially methylated regions

43 (DMRs). Annotatr and Chipenrich were used for genomic annotations and geneset enrichment tests of

44 DMRs, respe (DMRs). Annotatr and Chipenrich were used for genomic annotations and geneset enrichment tests of

44 DMRs, respectively.

45 Results: The cortex contained the majority of DMRs associated with Pb (69%) and DEHP (58%)

24 e
-
- 44 DMRs, respectively.
45 **Results:** The cortex c
46 exposure. The cortex
47 17 and 14 DMRs wit
48 males and females, re
49 regions associated wi
50 and exons). An analy
51 to be impacted by bot **Results:** The cortex contained the majority of DMRs associated with Pb (69%) and DEHP (58%) exposure. The cortex also contained the greatest degree of overlap in DMR signatures between sex 17 and 14 DMRs with Pb and DEHP
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- 46 exposure. The cortex also contained the greatest degree of overlap in DMR signatures between sexes (n = 17 and 14 DMRs with Pb and DEHP exposure, respectively) and exposure types (n = 79 and 47 DMRs in males and female
-
- 17 and 14 DMRs with Pb and DEHP exposure, respectively) and exposure types (n = 79 and 47 DMRs in

48 males and females, respectively). In all tissues, detected DMRs were preferentially found at genomic

49 regions associ males and females, respectively). In all tissues, detected DMRs were preferentially found at genomic

regions associated with gene expression regulation (e.g., CpG islands and shores, 5' UTRs, promoters

and exons). An ana For expression regulation (e.g., CpG islands and shores, 5' UTRs, promoters,

50 and exons). An analysis of GO terms associated with DMR-containing genes identified imprinted gene-

61 to be impacted by both Pb and DEHP e and exons). An analysis of GO terms associated with DMR-containing genes identified imprinted genes

51 to be impacted by both Pb and DEHP exposure. Of these, *Gnas* and *Grb10* contained DMRs across

52 tissues, sexes, an to be impacted by both Pb and DEHP exposure. Of these, *Gnas* and *Grb10* contained DMRs across
tissues, sexes, and exposures. DMRs were enriched in the imprinting control regions (ICRs) of *Gna.*
Grb10, with 15 and 17 I
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- 52 tissues, sexes, and exposures. DMRs were enriched in the imprinting control regions (ICRs) of *Gnas* and *Grb10*, with 15 and 17 ICR-located DMRs across cortex, blood, and liver in each gene, respectively. The ICRs were Grb10, with 15 and 17 ICR-located DMRs across cortex, blood, and liver in each gene, respectively. The

ICRs were also the location of DMRs replicated across target and surrogate tissues, suggesting epigenetic

changes the ICRs were also the location of DMRs replicated across target and surrogate tissues, suggesting epigenetic

changes these regions may be potentially viable biomarkers.
 Conclusions: We observed Pb- and DEHP-specific DNAm 55 changes these regions may be potentially viable biomarkers.
56 **Conclusions:** We observed Pb- and DEHP-specific DNAm c
greatest degree of overlap in DMR signatures was seen between
type. DNAm at imprinted control region **Conclusions:** We observed Pb- and DEHP-specific DNAm changes in cortex, blood, and liver, and the greatest degree of overlap in DMR signatures was seen between exposures followed by sex and tissue type. DNAm at imprinted greatest degree of overlap in DMR signatures was seen between exposures followed by sex and tissue

type. DNAm at imprinted control regions was altered by both Pb and DEHP, highlighting the

susceptibility of genomic impr type. DNAm at imprinted control regions was altered by both Pb and DEHP, highlighting the

susceptibility of genomic imprinting to these exposures during the perinatal window of develo

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 Introduction

The health impa
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- 59 susceptibility of genomic imprinting to these exposures during the perinatal window of development.

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 61 Introduction

62 The health impacts of toxicant exposures during early life, such as lead (Pb) and phthalate 61
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66 61 **Introduction**
62 The health import
64 (DOHaD) hyponduction
65 alter an organic maturation than
67 term by altering The health impacts of toxicant exposures during early life, such as lead (Pb) and phthalates (e.g., di(2-
ethylhexyl) phthalate, DEHP) can be framed within the Developmental Origins of Health and Disease
(DOHaD) hypothesi (DOHaD) hypothesis.¹ This hypothesis postulates that exposures during sensitive periods of development
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- term by altering the epigenome, which can have significant repercussions for health and disease.²
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- expression that are independent of the DNA sequence,³ with the most abundantly studied mechanism
- ethylhexyl) phthalate, DEHP) can be framed within the Developmental Origins of Health and Disease

(DOHaD) hypothesis.¹ This hypothesis postulates that exposures during sensitive periods of development

65 alter an orga 64 (DOHaD) hypothesis.¹ This hypothesis postulates that exposures during sensitive periods of development
65 alter an organism's normal developmental programming, triggering a myriad of effects on growth and
66 maturati
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- 65 alter an organism's normal developmental programming, triggering a myriad of effects on growth and
66 maturation that can persist into adulthood. Developmental exposures can impact gene expression long-
67 term by alte maturation that can persist into adulthood. Developmental exposures can impact gene expression long-

term by altering the epigenome, which can have significant repercussions for health and disease.²

Epigenetics refers 68901234r referred to as methylated cytosines (5mC), by DNA methyltransferases (DNMTs).⁴
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- and subsequent decreases in gene expression.⁵ Patterns of $5mC$ undergo waves of reprogramming (i.e.,
- Epigenetics refers to mitotically heritable and potentially reversible mechanisms modulating gene

69 expression that are independent of the DNA sequence,³ with the most abundantly studied mechani

70 being DNA methylat 69 expression that are independent of the DNA sequence,³ with the most abundantly studied mechanism

70 being DNA methylation (DNAm). DNAm entails the addition of a methyl group to the fifth position c

71 cytosine base 70 being DNA methylation (DNAm). DNAm entails the addition of a methyl group to the fifth position of cytosine base adjacent to a guanine (CpG, in the majority of cases), generating what are commonly referred to as methyl
- periods susceptible targets of developmental exposures.⁶
- 71 cytosine base adjacent to a guanine (CpG, in the majority of cases), generating what are commonly
72 referred to as methylated cytosines (5mC), by DNA methyltransferases (DNMTs).⁴ Increased levels
75 5 5 5 5 5 5 5 5 referred to as methylated cytosines (5mC), by DNA methyltransferases (DNMTs).⁴ Increased levels of
73 5mC within promoters and enhancers are typically associated with decreased transcription factor bindir
74 and subsequ 33 5mC within promoters and enhancers are typically associated with decreased transcription factor binding

27 and subsequent decreases in gene expression.⁵ Patterns of 5mC undergo waves of reprogramming (i.e.,

27 glob and subsequent decreases in gene expression.⁵ Patterns of 5mC undergo waves of reprogramming (i.e., global demethylation and remethylation) during critical windows of *in utero* development, making thes periods suscepti global demethylation and remethylation) during critical windows of *in utero* development, making these
periods susceptible targets of developmental exposures.⁶
Tight epigenetic regulation of imprinted genes is critical 77
78 Tight epigenetic regulation of imprinted genes is critical for early growth and development.^{7,8} Tight epigenetic regulation of imprinted genes is critical for early growth and development.^{7,8} Imprinted genes are expressed in a mono-allelic fashion, determined in a parent-of-origin manner. For instance, a genes are 78 genes are expressed in a mono-allelic fashion, determined in a parent-of-origin manner. For instance, a

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- stages are important during growth and early development.^{9,10}
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- The paternally expressed gene will contain an active paternal allele and an inactive (e.g., methylated and thus

So imprinted) maternal allele. The DNAm patterns of imprinted genes expressed at specific developmental

Sta maternal allele. The DNAm patterns of imprinted genes expressed at specific developmental

81 stages are important during growth and early development.^{9,10} Once DNAm patterns have been

82 established for these genes, o stages are important during growth and early development.^{9,10} Once DNAm patterns have been
established for these genes, often within imprinting control regions (ICRs) in gametes, they are
maintained through fertilizatio maintained through fertilization and extensive epigenetic reprogramming events.^{11,12} The specificity required to maintain patterns of genomic imprinting and re-establish DNAm in a parent-of-origin manner
-
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- been associated with changes in imprinted gene regulation and adverse health outcomes.^{13,14}
- established for these genes, often within imprinting control regions (ICRs) in gametes, they are

83 maintained through fertilization and extensive epigenetic reprogramming events.^{11,12} The specif

84 required to mainta
- maintained through fertilization and extensive epigenetic reprogramming events.^{11,12} The specificity

84 required to maintain patterns of genomic imprinting and re-establish DNAm in a parent-of-origin ma

85 following w 94 required to maintain patterns of genomic imprinting and re-establish DNAm in a parent-of-origin manner

95 following waves of global demethylation make gestational periods particularly sensitive to environmental

96 ex 85 following waves of global demethylation make gestational periods particularly sensitive to environmental
86 exposures. Environmentally-induced disruption of epigenetic processes during early development have
87 been as 86 exposures. Environmentally-induced disruption of epigenetic processes during early development have

87 been associated with changes in imprinted gene regulation and adverse health outcomes.^{13,14}

88 A variety of env 88901
88901
9933 88 A variety of environmental exposures, including Pb and DEHP, have been associated with altered patterns
89 of DNAm in humans and mice.^{15,16} Pb is a known neurotoxicant, with developmental exposures linked to
90 neuro of DNAm in humans and mice.^{15,16} 89 of DNAm in humans and mice.^{15,16} Pb is a known neurotoxicant, with developmental exposures linked to
90 neurological damage and cognition deficits in early life, as well as with increased risk of degenerative
91 neur
-
- neurological disease later in life.¹⁵ Although blood lead levels (BLLs) within the U.S. population have fallen dramatically, nearly 94% between 1976-1980 and 2015-2016, there is still concern regarding
- chronic low-levels of Pb exposure.¹⁷ This is especially true for early life exposures, as the developing
-
- brain and other organ systems are particularly susceptible to the toxic effects of Pb.¹⁸ Common sources of Pb exposure continue to be contaminated drinking water from leaded pipes as well as dust and chipping
- paint in older homes.^{19,20}
- 90 neurological damage and cognition deficits in early life, as well as with increased risk of degenerative
91 neurological disease later in life.¹⁵ Although blood lead levels (BLLs) within the U.S. population have
92 f 91 neurological disease later in life.¹⁵ Although blood lead levels (BLLs) within the U.S. population have
92 fallen dramatically, nearly 94% between 1976-1980 and 2015-2016, there is still concern regarding
93 chronic 92 fallen dramatically, nearly 94% between 1976-1980 and 2015-2016, there is still concern regarding

93 chronic low-levels of Pb exposure.¹⁷ This is especially true for early life exposures, as the developin

94 brain 23 chronic low-levels of Pb exposure.¹⁷ This is especially true for early life exposures, as the developing

24 brain and other organ systems are particularly susceptible to the toxic effects of Pb.¹⁸ Common source

2 94 brain and other organ systems are particularly susceptible to the toxic effects of Pb.¹⁸ Common sources of
95 Pb exposure continue to be contaminated drinking water from leaded pipes as well as dust and chipping
96 p 95 Pb exposure continue to be contaminated drinking water from leaded pipes as well as dust and chipping
96 paint in older homes.^{19,20} Exposure to DEHP, a phthalate commonly used as a plasticizer, has become
97 ubiquito 96 paint in older homes.^{19,20} Exposure to DEHP, a phthalate commonly used as a plasticizer, has become ubiquitous, with most U.S. adults having detectable levels of DEHP metabolites in their urine.²¹ DEHI a known endo ubiquitous, with most U.S. adults having detectable levels of DEHP metabolites in their urine.²¹ DEHP is
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- 97 ubiquitous, with most U.S. adults having detectable levels of DEHP metabolites in their urine.²¹ DEHP is
98 a known endocrine disruptor, with developmental exposures associated with altered metabolic
99 function.^{16,} 28 a known endocrine disruptor, with developmental exposures associated with altered metabolic

29 function.^{16,22} Common routes of DEHP exposure include personal care products, food and beve

20 containers, and medical function. $16,22$ Common routes of DEHP exposure include personal care products, food and beverage
- containers, and medical equipment, making gestational and developmental exposures common.²³ Despite
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- 99 function.^{16,22} Common routes of DEHP exposure include personal care products, food and beverage

00 containers, and medical equipment, making gestational and developmental exposures common.²³ De

91 great progress 100 containers, and medical equipment, making gestational and developmental exposures common.²⁵ Despite

101 great progress over the years, gaps in knowledge remain as to whether perinatal Pb or DEHP exposure-

102 medi
- Transcription (TaRGET II) Consortium,²⁴
-
- 101 great progress over the years, gaps in knowledge remain as to whether perinatal Pb or DEHP exposure-

102 mediated changes in DNAm have implications for long-term disease risk, whether there are sex-specific

103 effec 103 effects, and if these changes are conserved among tissues.

104 As a part of the Toxicant Exposures and Responses by Ger

105 Transcription (TaRGET II) Consortium,²⁴ we utilized a mo

106 DEHP exposures to investigat 104 As a part of the Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of

105 Transcription (TaRGET II) Consortium,²⁴ we utilized a mouse model of human-relevant perinat

106 DEHP exposures to invest 106 DEHP exposures to investigate genome-wide tissue- and sex-specific associations with changes in

107 DNAm. Whole genome bisulfite sequencing (WGBS) quantified DNAm changes in blood (an easil

108 accessible and therefo
- mediated changes in DNAm have implications for long-term disease risk, whether there are sex-specific

103 effects, and if these changes are conserved among tissues.

104 As a part of the Toxicant Exposures and Responses b
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- Transcription (TaRGET II) Consortium,²⁴ we utilized a mouse model of human-relevant perinatal Pb and
106 DEHP exposures to investigate genome-wide tissue- and sex-specific associations with changes in
107 DNAm. Whole gen 107 DNAm. Whole genome bisulfite sequencing (WGBS) quantified DNAm changes in blood (an easily

108 accessible and therefore considered a "surrogate" tissue) as well as cortex and liver (two tissues often

109 difficult to 108 accessible and therefore considered a "surrogate" tissue) as well as cortex and liver (two tissues often

109 difficult to access, representing "target" tissues) collected from male and female 5-month-old mice, w

110 difficult to access, representing "target" tissues) collected from male and female 5-month-old mice, with

110 and without perinatal Pb or DEHP exposures. We assessed whether perinatal Pb- or DEHP-exposed mice

111 display 110 and without perinatal Pb or DEHP exposures. We assessed whether perinatal Pb- or DEHP-exposed mice

111 displayed changes in DNAm across the genome and identified imprinted genes as a relevant gene class

112 common to 111 displayed changes in DNAm across the genome and identified imprinted genes as a relevant gene class

112 common to these two exposures. We additionally tested whether DNAm patterns in the surrogate tissue

113 (blood)
-
- 112 common to these two exposures. We additionally tested whether DNAm patterns in the surrogate tissue

113 (blood) correlated with those seen in target tissues, to determine if blood provides a viable signature for

114 (blood) correlated with those seen in target tissues, to determine if blood provides a viable signature for

114 Pb- or DEHP-induced epigenetic changes in these two tissues, and how these patterns differed between

115 Met
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114 Pb- or DEHP-induced epigenetic changes in these two tissues, and how these patterns differed between
115 males and females.
116 **Methods**
117 *Animal exposure paradigm and tissue collection*
118 Wild-type non-agouti 115 males and females.

116 **Methods**

117 Animal exposure pa

118 Wild-type non-agou

119 (A^{vy}) mice, which as 116 **Methods**

117 *Animal exp*

118 Wild-type

119 (A^{vy}) mice,

120 $(6-8 \text{ weeks})$

121 mating with

 (A^{vy}) mice, which are genetically invariant and 93% identical to the C57BL/6J strain.²⁵

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- 117 *Animal exposure paradigm and tissue collection*

118 Wild-type non-agouti a/a mice were obtained fro

119 (A^{vy}) mice, which are genetically invariant and 93

120 (6-8 weeks old) were randomly assigned to contr

121
- 118 Wild-type non-agouti *a/a* mice were obtained from an over 230-generation colony of viable yellow agouti

119 ($A^{(v)}$) mice, which are genetically invariant and 93% identical to the C57BL/6J strain.²⁵ Virgin *a/a* 119 ($A^{(v)}$) mice, which are genetically invariant and 93% identical to the C57BL/6J strain.²⁵ Virgin a/a females (6-8 weeks old) were randomly assigned to control, Pb-acetate water, or DEHP-chow two weeks prior to ma 120 (6-8 weeks old) were randomly assigned to control, Pb-acetate water, or DEHP-chow two weeks prior to mating with virgin a/a males (7-9 weeks old). Pb- and DEHP-exposure were conducted *ad libitum* via distilled drink 121 mating with virgin a/a males (7-9 weeks old). Pb- and DEHP-exposure were conducted *ad libitum* via
122 distilled drinking water mixed with Pb-acetate or 7% corn oil chow mixed with DEHP. The Pb-acetate
123 concentra 122 distilled drinking water mixed with Pb-acetate or 7% corn oil chow mixed with DEHP. The Pb-acetate
123 concentration was set as 32ppm to model human relevant perinatal exposure, where we have previously
124 measured m 123 concentration was set as 32ppm to model human relevant perinatal exposure, where we have previously
124 measured murine maternal BLLs around 16-60 ug/dL (mean: 32.1 ug/dL).²⁶ DEHP was dissolved in corn
124 measured measured murine maternal BLLs around 16-60 ug/dL (mean: 32.1 ug/dL).²⁶ DEHP was dissolved in corn measured murine maternal BLLs around 16-60 ug/dL (mean: 32.1 ug/dL).²⁶ DEHP was dissolved in corn $\frac{2}{3}$

125 oil from Envigo to create a customized stock solution, to produce 7% corn oil chow for experimentation.

126 The DEHP exposure level was selected based on a target maternal dose of 5 mg/kg-day and assumes that

127 a 126 The DEHP exposure level was selected based on a target maternal dose of 5 mg/kg-day and assumes that

127 a pregnant and nursing female mice weighs approximately 25 g and ingests roughly 5 g of chow per day.

128 This 127 a pregnant and nursing female mice weighs approximately 25 g and ingests roughly 5 g of chow per day.

128 This target dose was selected as previous literature demonstrates obesity-related phenotypes in offspring

129 128 This target dose was selected as previous literature demonstrates obesity-related phenotypes in offspring

129 exposed to 5 mg/kg-day DEHP during early development,^{22,27} and this dosage falls within the range of

13 exposed to 5 mg/kg-day DEHP during early development,^{22,27} and this dosage falls within the range of 129 exposed to 5 mg/kg-day DEHP during early development,^{22,27} and this dosage falls within the range of exposures previously documented in humans.²⁸ All animals were maintained on a phytoestrogen-free modified AIN-93 exposures previously documented in humans.²⁸ 130 exposures previously documented in humans.²⁸ All animals were maintained on a phytoestrogen-free modified AIN-93 G diet (Td.95092, 7% corn oil diet, Envigo) while housed in polycarbonate-free cage.

132 Animal expos 131 modified AIN-93 G diet (Td.95092, 7% corn oil diet, Envigo) while housed in polycarbonate-free cages.

132 Animal exposure to Pb or DEHP continued through gestation and lactation until weaning at post-natal da

133 21 132 Animal exposure to Pb or DEHP continued through gestation and lactation until weaning at post-natal day

133 21 (PND21) when pups were switched to either Pb-free drinking water or DEHP-free chow. Perinatal

134 exposu 133 21 (PND21) when pups were switched to either Pb-free drinking water or DEHP-free chow. Perinatal

134 exposure, thus, occurred in offspring throughout fetal development and the first three weeks after birth

135 Offsp 134 exposure, thus, occurred in offspring throughout fetal development and the first three weeks after birth.

135 Offspring were maintained until 5 months of age. This study included $n \ge 5$ males and $n \ge 5$ females for 135 Offspring were maintained until 5 months of age. This study included n ≥ 5 males and n ≥ 5 females for

136 Pb-exposed, DEHP-exposed, and control groups, each containing 1 male and 1 female mouse per litter;

137 and 136 Pb-exposed, DEHP-exposed, and control groups, each containing 1 male and 1 female mouse per litter;

137 and a final samples size of $n = 108$ once tissues (i.e., cortex, blood, and liver) were collected. All animal

1 137 and a final samples size of n = 108 once tissues (i.e., cortex, blood, and liver) were collected. All animals

138 and collected tissues were included in subsequent analyses, with no exclusions necessary. Prior to

13 and collected tissues were included in subsequent analyses, with no exclusions necessary. Prior to

139 euthanasia, mice were fasted for 4 hours during the light cycle beginning in the morning, with eut

140 and tissue col 2139 euthanasia, mice were fasted for 4 hours during the light cycle beginning in the morning, with euthanasia

2140 asphyxiation, blood was collected through cardiac puncture, followed by dissection of the cortex and

21 and tissue collection occurring in the afternoon. Immediately following mouse euthanasia with CO₂

141 asphyxiation, blood was collected through cardiac puncture, followed by dissection of the cortex an

142 liver, which 141 asphyxiation, blood was collected through cardiac puncture, followed by dissection of the cortex and

142 liver, which were immediately flash frozen in liquid nitrogen and stored at -80°C. Animal collection v

143 sta 142 liver, which were immediately flash frozen in liquid nitrogen and stored at -80°C. Animal collection was

143 standardized to between 1pm to 3pm and collection order was randomized daily. For each mouse, one

144 inve transfer 1914 standardized to between 1pm to 3pm and collection order was randomized daily. For each mouse, one

144 investigators (KN) administered the treatment and was therefore aware of the treatment group allocation
 144 investigator (KN) administered the treatment and was therefore aware of the treatment group allocation.

145 All investigators completing subsequent molecular assays were blinded to treatment group, until

146 treatme 145 All investigators completing subsequent molecular assays were blinded to treatment group, until
146 treatment group was analyzed during bioinformatic analyses. All mouse procedures were approve
147 University of Michig treatment group was analyzed during bioinformatic analyses. All mouse procedures were approved by the

147 University of Michigan Institutional Animal Care and Use Committee (IACUC), and animals were treated

148 humanely University of Michigan Institutional Animal Care and Use Committee (IACUC), and animals were treated

148 humanely and with respect. All experiments were conducted according to experimental procedures

149 outlined by the outlined by the NIEHS TaRGET II Consortium.²⁴ In drafting this manuscript, ARRIVE reporting guidelines were used to ensure quality and transparency of reported work.²⁹

248 humanely and with respect. All experiments were conducted according to experimental procedures

149 outlined by the NIEHS TaRGET II Consortium.²⁴ In drafting this manuscript, ARRIVE reporting

150 guidelines were use 249 outlined by the NIEHS TaRGET II Consortium.²⁴ In drafting this manuscript, ARRIVE reporting

250 guidelines were used to ensure quality and transparency of reported work.²⁹

251 *DNA extraction and whole genome bi* 150
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156 151 *DNA extraction and whole genome bisulfite sequencing*
152 DNA extraction was performed using the AllPrep DNA/
153 #80224). Additional details about the animal exposures,
154 be found in previously published protocols. 152 DNA extraction was performed using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, Cat.

153 #80224). Additional details about the animal exposures, blood collection, and blood DNA extraction

154 be found in previous 480224). Additional details about the animal exposures, blood collection, and blood DNA extraction can

154 be found in previously published protocols.³⁰ Genomic DNA (gDNA) was used in the preparation of

155 WGBS libra be found in previously published protocols.³⁰ Genomic DNA (gDNA) was used in the preparation of 154 be found in previously published protocols.³⁰ Genomic DNA (gDNA) was used in the preparation of
155 WGBS libraries at the University of Michigan Epigenomics Core. gDNA was quantified using the Qt
156 BR dsDNA kit (Fi WGBS libraries at the University of Michigan Epigenomics Core. gDNA was quantified using the Qubit

156 BR dsDNA kit (Fisher, Cat. #Q32850), and quality assessed using Agilent's Genomic DNA Tapestation

157 Kit (Agilent, C 156 BR dsDNA kit (Fisher, Cat. #Q32850), and quality assessed using Agilent's Genomic DNA Tapestation

157 Kit (Agilent, Cat. #A63880). For each sample, 200 ng of gDNA was spiked with 0.5% of unmethylated

158 lambda DNA 157 Kit (Agilent, Cat. #A63880). For each sample, 200 ng of gDNA was spiked with 0.5% of unmethylated

158 lambda DNA and sheared using a Covaris S220 (10% Duty Factor, 140W Peak Incident Power, 200

159 Cycle/Burst, 55s) 158 lambda DNA and sheared using a Covaris S220 (10% Duty Factor, 140W Peak Incident Power, 200
159 Cycle/Burst, 55s). A 2 μl aliquot of processed gDNA was taken to assess shearing using an Agilent I
160 Sensitivity D1000 159 Cycle/Burst, 55s). A 2 µl aliquot of processed gDNA was taken to assess shearing using an Agilent High

160 Sensitivity D1000 Kit (Agilent, Cat. #G2991AA). Once shearing was assessed, the remaining gDNA was

161 conce 160 Sensitivity D1000 Kit (Agilent, Cat. #G2991AA). Once shearing was assessed, the remaining gDNA was

161 concentrated using a Qiagen PCR Purification column and processed for end-repair and A-tailing.

162 Ligation of 161 concentrated using a Qiagen PCR Purification column and processed for end-repair and A-tailing.

162 Ligation of cytosine-methylated adapters was done overnight at 16°C. Following this, ligation process were cleaned us Ligation of cytosine-methylated adapters was done overnight at 16°C. Following this, ligation products

163 were cleaned using AMPure XP Beads (Fisher, Cat. #NC9933872) before processing for bisulfite

164 conversion using were cleaned using AMPure XP Beads (Fisher, Cat. #NC9933872) before processing for bisulfite

164 conversion using the Zymo EZ DNA Methylation Kit (Zymo, Cat. #D5001), and by amplifying th

165 bisulfite converted products 164 conversion using the Zymo EZ DNA Methylation Kit (Zymo, Cat. #D5001), and by amplifying the

165 bisulfite converted products over 55 cycles of 95°C for 30 seconds followed by 55°C for 15 minutes

166 according to the 165 bisulfite converted products over 55 cycles of 95°C for 30 seconds followed by 55°C for 15 minutes,

166 according to the manufacturer's guidelines. After cleanup of the bisulfite converted products, final

167 librari according to the manufacturer's guidelines. After cleanup of the bisulfite converted products, final

167 libraries were amplified over 10 cycles by PCR using KAPA Uracil+ Ready Mix (Fisher, Cat.

168 #501965287) and NEB d 167 libraries were amplified over 10 cycles by PCR using KAPA Uracil+ Ready Mix (Fisher, Cat.
168 #501965287) and NEB dual indexing primers. Final libraries were cleaned with AMPure XP b
169 concentration assessed using th 4501965287) and NEB dual indexing primers. Final libraries were cleaned with AMPure XP beads,
169 concentration assessed using the Qubit BR dsDNA Kit and library size assessed on the Agilent High
170 Sensitivity D1000 Tape 169 concentration assessed using the Qubit BR dsDNA Kit and library size assessed on the Agilent High
170 Sensitivity D1000 Tapestation Kit. Prior to pooling, each library was quantified using KAPA Library
171 Quantificati 170 Sensitivity D1000 Tapestation Kit. Prior to pooling, each library was quantified using KAPA Library
171 Quantification Kit (Fisher, Cat. #501965234). We constructed four different pools of 18 libraries and
172 pool was 171 Quantification Kit (Fisher, Cat. #501965234). We constructed four different pools of 18 libraries and each
172 pool was sequenced on an Illumina NovaSeq6000 S4 200 cycle flow cell (PE-100) at the University of
173 Mich 172 pool was sequenced on an Illumina NovaSeq6000 S4 200 cycle flow cell (PE-100) at the University of
173 Michigan Advanced Genomics Core. Unless otherwise stated, all enzymes used in library generation we
173 Michigan Ad 173 Michigan Advanced Genomics Core. Unless otherwise stated, all enzymes used in library generation were
4

174 purchased from New England Biolabs. Adapters with universally methylated cytosines were synthesized

175 by Integrated DNA Technologies (IDT).

176 *Data processing, quality control, and differential DNA methylation a* 175 by Integrated DNA Technologies (IDT).

176 *Data processing, quality control, and diff*

177 FastQC³¹ (v0.11.5) and MultiQC³² (v1.8)

178 Sequencing adapters and low-quality bas

179 reads shorter than 20 bp were 2176 *Data processing, quality control, and differential DNA methylation analysis*

2177 FastQC³¹ (v0.11.5) and MultiQC³² (v1.8) were used to assess the quality of a

2178 Sequencing adapters and low-quality bases wer FastQC 31 (v0.11.5) and MultiQC 32 177 FastQC³¹ (v0.11.5) and MultiQC³² (v1.8) were used to assess the quality of all sequenced samples.

178 Sequencing adapters and low-quality bases were removed by Trim Galore³³ (v0.4.5). After trimmin

179 reads s Sequencing adapters and low-quality bases were removed by Trim Galore³³ 178 Sequencing adapters and low-quality bases were removed by Trim Galore³³ (v0.4.5). After trimming,

179 reads shorter than 20 bp were removed from further analysis. Bismark³⁴ (v0.19.0) with Bowtie 2³⁵

180 (v2.3. reads shorter than 20 bp were removed from further analysis. Bismark³⁴ (v0.19.0) with Bowtie 2^{35} $\begin{array}{c} 179 \\ 180 \\ 181 \\ 182 \\ 183 \\ 184 \\ 185 \\ 186 \\ 187 \end{array}$ 180 (v2.3.4) as backend alignment software were used for read alignment and methylation calling with

181 Genome Reference Consortium Mouse Build 38 (mm10) as the reference genome. All alignments

182 performed with 0 mis 181 Genome Reference Consortium Mouse Build 38 (mm10) as the reference genome. All alignments were

182 performed with 0 mismatches and multi-seed length of 20 bp. The bisulfite conversion rates were

183 calculated throu 182 performed with 0 mismatches and multi-seed length of 20 bp. The bisulfite conversion rates were

183 calculated through the unmethylated lambda phage DNA spike-ins. Metilene³⁶ (v0.2.8) and R

184 Bioconductor packag calculated through the unmethylated lambda phage DNA spike-ins. Metilene³⁶ (v0.2.8) and R 183 calculated through the unmethylated lambda phage DNA spike-ins. Metilene³⁶ (v0.2.8) and R
184 Bioconductor package methylSig³⁷ (v1.4.0) were used to identify the differentially methylated
185 (DMRs) independently. Bioconductor package methyl Sig^{37} (v1.4.0) were used to identify the differentially methylated regions 184 Bioconductor package methylSig³⁷ (v1.4.0) were used to identify the differentially methylated regions

185 (DMRs) independently. CpG sites with less than 10 reads or more than 500 reads were excluded from

186 DMR d 185 (DMRs) independently. CpG sites with less than 10 reads or more than 500 reads were excluded from

186 DMR detection. For methylSig, CpG sites that had reads covered in fewer than 4 samples within a

187 treatment gro 186 DMR detection. For methylSig, CpG sites that had reads covered in fewer than 4 samples within a
187 treatment group were filtered out for DMR identification. Tiling windows were used with methylSi
188 identify DMRs, w 187 treatment group were filtered out for DMR identification. Tiling windows were used with methylSig to

188 identify DMRs, with a window size of 100 bp. For metilene, DMRs were identified *de novo* with at leas

189 5 C 188 identify DMRs, with a window size of 100 bp. For metilene, DMRs were identified *de novo* with at least

189 5 CpGs in a single DMR. For both methods, an FDR cutoff of < 0.15 and a DNAm difference of >5%

190 were app 189 5 CpGs in a single DMR. For both methods, an FDR cutoff of < 0.15 and a DNAm difference of $>5\%$ were applied to select significant DMRs. All overlapping DMRs from methylSig and metilene were confirmed to be in t 190 were applied to select significant DMRs. All overlapping DMRs from methylSig and metilene were

191 confirmed to be in the same direction and merged for downstream analysis (**Supplementary Table** 1

192 minimum overla 191 confirmed to be in the same direction and merged for downstream analysis (**Supplementary Table 1**). A minimum overlap cutoff of \geq 10bp was applied to identify overlapping DMRs between tissues, sexes, and exposures 192 minimum overlap cutoff of ≥ 10bp was applied to identify overlapping DMRs between tissues, sexes, and
193 exposures, based on DMR coordinates, with no specification of methylation change direction considered
194 for exposures, based on DMR coordinates, with no specification of methylation change direction considered
194 for the purposes of initial comparisons. The annotatr Bioconductor package³⁸ was used to annotate all
195 signific for the purposes of initial comparisons. The annotatr Bioconductor package³⁸ was used to annotate all 194 for the purposes of initial comparisons. The annotatr Bioconductor package³⁸ was used to annotate all
195 significant DMRs associated with genes and genomic locations, including CpG islands, CpG shores, C
196 shelves 195 significant DMRs associated with genes and genomic locations, including CpG islands, CpG shores, CpG shelves, promoters, exons, introns, 5' UTRs, 3' UTRs, enhancers, and regions 1-5kb upstream of transcription start si 196 shelves, promoters, exons, introns, 5' UTRs, 3' UTRs, enhancers, and regions 1-5kb upstream of

197 transcription start sites (TSSs). Random genomic regions were generated and annotated with anno

198 each tissue usin 197 transcription start sites (TSSs). Random genomic regions were generated and annotated with annotatr for

198 each tissue using the mm10 reference genome. These random regions were used as background

199 information t 208 each tissue using the mm10 reference genome. These random regions were used as background

200 information to show the distribution of the genomic annotation of the DMRs if distributed purel

201 chance. An overview o

199 information to show the distribution of the genomic annotation of the DMRs if distributed purely by

200 chance. An overview of the complete methods is illustrated in **Figure 1**.

201 *Geneset enrichment test*

202 R R Bioconductor package Chipenrich³⁹

-
-
- chance. An overview of the complete methods is illustrated in **Figure 1.**

201 *Geneset enrichment test*

202 R Bioconductor package Chipenrich³⁹ (v2.16.0) was used to perform gen

203 Ontology (GO) terms enriched with s 201 *Geneset enrichment test*

202 R Bioconductor package

203 Ontology (GO) terms en

204 each tissue and sex (i.e.,

205 liver) across each exposure

206 the *nearest_tss* locus def

207 Biological Process (BP),
-
-
- 202 R Bioconductor package Chipenrich³⁹ (v2.16.0) was used to perform gene set enrichment testing of Gene
203 Ontology (GO) terms enriched with significant DMRs. Twelve analyses were performed stratified by
204 each tis 203 Ontology (GO) terms enriched with significant DMRs. Twelve analyses were performed stratified by

204 each tissue and sex (i.e., male cortex, male blood, male liver, female cortex, female blood, and female

205 liver) 204 each tissue and sex (i.e., male cortex, male blood, male liver, female cortex, female blood, and female

205 liver) across each exposure group (i.e., Pb, DEHP, and control). Gene assignments were determined w

206 the 205 liver) across each exposure group (i.e., Pb, DEHP, and control). Gene assignments were determined with

206 the *nearest_tss* locus definition in the *chipenrich* function to find all three categories of ontology (i.e. 206 the *nearest_tss* locus definition in the *chipenrich* function to find all three categories of ontology (i.e.,
207 Biological Process (BP), Cellular Component (CC), and Molecular Function (MF)). An FDR cutoff of
208 0 207 Biological Process (BP), Cellular Component (CC), and Molecular Function (MF)). An FDR cutoff of $<$ 208 0.05 was applied for selecting significantly enriched GO terms. GO terms containing fewer than 15 genes or more 208 0.05 was applied for selecting significantly enriched GO terms. GO terms containing fewer than 15 genes

209 or more than 500 genes were removed from analysis.

210 *Mouse imprinted genes and imprinted control regions*

- 209 or more than 500 genes were removed from analysis.

210 *Mouse imprinted genes and imprinted control regions*

211 DMRs were compared to mouse imprinted genes and

212 genes using previously documented efforts^{40–42} 210 *Mouse imprinted genes and imprinted control regions*
211 DMRs were compared to mouse imprinted genes and I
212 genes using previously documented efforts^{40–42} and obl
213 R package⁴⁴ (0.6.4) was used to identify ov
- 211 DMRs were compared to mouse imprinted genes and ICRs. We compiled a reference list of imprinted
212 genes using previously documented efforts⁴⁰⁻⁴² and obtained ICR coordinates from Wang et al.⁴³ The v
213 R packag genes using previously documented efforts^{40–42} and obtained ICR coordinates from Wang et al.⁴³ The valr

213 R package⁴⁴ (0.6.4) was used to identify overlapping regions between the DMRs and ICRs. A Binomial

214 te
- R package⁴⁴ (0.6.4) was used to identify overlapping regions between the DMRs and ICRs. A Binomial
- 213 R package⁴⁴ (0.6.4) was used to identify overlapping regions between the DMRs and ICRs. A Binomial
214 test was used to assess whether the DMRs were significantly enriched in ICRs and an adjusted p-value <
215 0.05 214 test was used to assess whether the DMRs were significantly enriched in ICRs and an adjusted p-value < 0.05 cutoff was utilized for identifying significant results.
216 Results
- 215 0.05 cutoff was utilized for identifying significant results.
216 **Results**
- 216 **Results**

217 *Differentially methylated regions among perinatally Pb- and DEHP-exposed tissues*
218 Among Pb-exposed tissues, the majority of the DMRs were detected in the cortex (m
219 female (F) = 746), followed by blood (M = 24 218 Among Pb-exposed tissues, the majority of the DMRs were detected in the cortex (male (M) = 688,

219 female (F) = 746), followed by blood (M = 243, F = 292), and liver (M = 100, F = 36). A similar pat

220 was observe 219 female (F) = 746), followed by blood (M = 243, F = 292), and liver (M = 100, F = 36). A similar pattern
220 was observed in DEHP-exposed tissues, with the majority of DMRs detected in the cortex (M = 587, F =
221 661) 220 was observed in DEHP-exposed tissues, with the majority of DMRs detected in the cortex (M = 587, F = 221 661), followed by blood (M = 312, F = 477), and liver (M = 90, F = 40) (**Figure 2A**). There was limited overlap 221 661), followed by blood ($M = 312$, $F = 477$), and liver ($M = 90$, $F = 40$) (**Figure 2A**). There was limited
222 overlap in DMRs between each tissue type, relative to the total number detected in each tissue and sex
22 overlap in DMRs between each tissue type, relative to the total number detected in each tissue and sex
 223 (Figure 2B). For instance, Pb-exposed animals had only few DMRs appear in multiple tissues. Males 1

224 3 commo **Figure 2B**). For instance, Pb-exposed animals had only few DMRs appear in multiple tissues. Males had

224 3 common DMRs among all three tissues, with 5 DMRs each overlapping between cortex and blood,

225 between cortex 224 3 common DMRs among all three tissues, with 5 DMRs each overlapping between cortex and blood,

225 between cortex and liver, and between liver and blood. Females had 7 common DMRs between corter

226 and blood, 3 betwe 225 between cortex and liver, and between liver and blood. Females had 7 common DMRs between cortex

226 and blood, 3 between cortex and liver, and 1 between liver and blood, with no DMRs detected in all the

227 issues. S 226 and blood, 3 between cortex and liver, and 1 between liver and blood, with no DMRs detected in all three

227 tissues. Similar patterns were presented in DEHP-exposed animals, wherein males had 1 DMR common

228 to all 227 tissues. Similar patterns were presented in DEHP-exposed animals, wherein males had 1 DMR common

228 to all three tissues and 10 detected in cortex and blood, and no overlap among the remaining tissue pairs.

229 DEHP to all three tissues and 10 detected in cortex and blood, and no overlap among the remaining tissue pairs.

229 DEHP-exposed females had more overlapping DMRs compared to males, with 2 DMR common to all

230 tissues, 13 in DEHP-exposed females had more overlapping DMRs compared to males, with 2 DMR common to all

230 tissues, 13 in both cortex and blood, 5 in cortex and liver, and 3 in liver and blood (**Figure 2B**).

231 Relative to the low tissues, 13 in both cortex and blood, 5 in cortex and liver, and 3 in liver and blood (**Figure 2B**).

231 Relative to the low overlap in exposure associated DMRs between tissues, there was more DMR

233 similarity between 231
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239 Relative to the low overlap in exposure associated DMRs between tissues, there was more DMR

233 similarity between the sexes when stratified by tissue (**Figure 2C**), with the exception of the liver

234 exposed animals, 1 233 similarity between the sexes when stratified by tissue (**Figure 2C**), with the exception of the liver. In Pb-
234 exposed animals, 17 and 10 DMRs were common to both males and females in the cortex and blood,
235 respe exposed animals, 17 and 10 DMRs were common to both males and females in the cortex and blood,

235 respectively. Similarly, in DEHP-exposed animals, 14 and 11 DMRs were found in both males and

236 females in the cortex a respectively. Similarly, in DEHP-exposed animals, 14 and 11 DMRs were found in both males and

236 females in the cortex and blood, respectively (**Figure 2C**). Overall, the greatest degree of DMR ove

237 was found between 236 females in the cortex and blood, respectively (**Figure 2C**). Overall, the greatest degree of DMR overlap

237 was found between exposure types. Pb- and DEHP-exposed cortex has the greatest degree of overlap,

238 with

237 was found between exposure types. Pb- and DEHP-exposed cortex has the greatest degree of overlap,

238 with 79 and 47 DMRs detected under both exposure conditions in males and females, respectively

239 (Figure 2D). 29 238 with 79 and 47 DMRs detected under both exposure conditions in males and females, respectively

239 (Figure 2D). 29 and 28 DMRs appeared in both exposure conditions in male and female blood,

240 respectively, whereas **Example 2D**). 29 and 28 DMRs appeared in both exposure conditions in male and female blood, respectively, whereas Pb- and DEHP-exposed liver shared 2 DMRs in each sex (**Figure 2D**). 241 Patterns in the direction of DNA me expectively, whereas Pb- and DEHP-exposed liver shared 2 DMRs in each sex (**Figure 2D**).

241 Patterns in the direction of DNA methylation changes (DNA hyper or hypomethylation) were

243 and exposure specific (**Figure 2E** 241
242
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249 242 Patterns in the direction of DNA methylation changes (DNA hyper or hypomethylation) were tissue, sex,

243 and exposure specific (**Figure 2E**). Among Pb- and DEHP-exposed cortex, the majority of DMRs

244 detected in 243 and exposure specific (**Figure 2E**). Among Pb- and DEHP-exposed cortex, the majority of DMRs

244 detected in males and females were hypomethylated, with slightly greater rates of hypomethylatior

245 in males (Pb male 244 detected in males and females were hypomethylated, with slightly greater rates of hypomethylation seen

245 in males (Pb male = 80%, Pb female = 52%, DEHP male = 60%, DEHP female = 58%). DMRs in Pb-

246 exposed femal 245 in males (Pb male = 80%, Pb female = 52%, DEHP male = 60%, DEHP female = 58%). DMRs in Pb-
246 exposed female blood, as well as DEHP-exposed male and female blood, tended to be hypermethylate
247 (Pb female = 71%, DEH 246 exposed female blood, as well as DEHP-exposed male and female blood, tended to be hypermethylated

247 (Pb female = 71%, DEHP male = 63%, DEHP female = 64%). In contrast, among Pb-exposed male

248 blood, 56% of DMRs w 247 (Pb female = 71%, DEHP male = 63%, DEHP female = 64%). In contrast, among Pb-exposed male

248 blood, 56% of DMRs were hypomethylated. Patterns of directionality were more distinct between

249 exposure types in the l 248 blood, 56% of DMRs were hypomethylated. Patterns of directionality were more distinct between

249 exposure types in the liver. Pb-exposed male liver presented a high proportion of hypermethylated

250 (66%), whereas P exposure types in the liver. Pb-exposed male liver presented a high proportion of hypermethylated DMRs (66%), whereas Pb-exposed female liver has slightly more hypomethylated DMRs (56%). DMR direction was roughly evenly sp 250 (66%), whereas Pb-exposed female liver has slightly more hypomethylated DMRs (56%). DMR direction

251 was roughly evenly split in DEHP-exposed liver, with 50% and 53% of DMRs hypermethylated in males

252 and females, 251 was roughly evenly split in DEHP-exposed liver, with 50% and 53% of DMRs hypermethylated in males

252 and females, respectively (**Figure 2E**). **Supplementary Table 1** provides a summary of all DMRs

253 detected in th

252 and females, respectively (**Figure 2E**). **Supplementary Table 1** provides a summary of all DMRs
253 detected in this analysis.
254 *Prevalence of detected DMRs in mouse genomic regions*
256 The DMRs detected in this st 253 detected in this analysis.

254

255 *Prevalence of detected D*

256 The DMRs detected in th

257 have been expected by a

258 sites in the mouse genom

259 mapped to CpG islands to 254
255
2567
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261 Prevalence of detected DMRs in mouse genomic regions

256 The DMRs detected in this study occurred in specific generated the property of the mouse genome (mm10). According to **Figure**

259 mapped to CpG islands to a greate 256 The DMRs detected in this study occurred in specific genomic regions to a greater degree than would

257 have been expected by a random distribution generated for comparison, given known patterns of CpG

258 sites in t 257 have been expected by a random distribution generated for comparison, given known patterns of CpG

258 sites in the mouse genome (mm10). According to **Figure 3** and **Supplementary Table 2**, detected DM

259 mapped to C 258 sites in the mouse genome (mm10). According to **Figure 3** and **Supplementary Table 2**, detected DMRs mapped to CpG islands to a greater degree than would have been expected by chance (3.37-19.07% of all DMRs across sex 259 mapped to CpG islands to a greater degree than would have been expected by chance (3.37-19.07% of all

260 DMRs across sex, tissues, and exposures, compared to 0.12-0.29% at random). In blood and cortex across

261 bot 260 DMRs across sex, tissues, and exposures, compared to 0.12-0.29% at random). In blood and cortex across
261 both sexes and exposures, more DMRs were detected in 5' UTRs than predicted 4.03-8.79%, compared to
262 0.18-0. 261 both sexes and exposures, more DMRs were detected in 5' UTRs than predicted 4.03-8.79%, compared to 0.18-0.4% under a random distribution), and a similar pattern was observed in liver of Pb-exposed males and females (2 262 0.18-0.4% under a random distribution), and a similar pattern was observed in liver of Pb-exposed males

263 and females (2.02-2.91%) as well as DEHP-exposed females (4.8%, compared to 0.29% under a random

264 distrib 263 and females (2.02-2.91%) as well as DEHP-exposed females (4.8%, compared to 0.29% under a random
264 distribution). Several transcriptional regulatory regions demonstrated significant derivation from what
265 would be 264 distribution). Several transcriptional regulatory regions demonstrated significant derivation from what
265 would be expected by chance as well. DMRs were present in promoter regions 2.83-6.61 times more the
265 265 would be expected by chance as well. DMRs were present in promoter regions 2.83-6.61 times more than 6

266 would have been predicted by chance across all conditions (7.3-14.41%, compared to 1.74-2.58% at random). Exons were another notable location of DMRs, with 1.76-4.99 times more DMRs than wha

268 would have been seen u 267 random). Exons were another notable location of DMRs, with 1.76-4.99 times more DMRs than what

268 would have been seen under a random distribution (6.74-18.65%, compared to 3.37-3.84% at random)

269 Conversely, ther 268 would have been seen under a random distribution (6.74-18.65%, compared to 3.37-3.84% at random).

269 Conversely, there were fewer DMRs detected in the open sea (11.02-21.13% in blood, 18.40-44.94% in

270 liver, and 269 Conversely, there were fewer DMRs detected in the open sea (11.02-21.13% in blood, 18.40-44.94% in

270 liver, and 19.91-25.87% in cortex) than would be expected by chance (54.56-58.09%) (**Figure 3,**

271 **Supplementar** 270 liver, and 19.91-25.87% in cortex) than would be expected by chance (54.56-58.09%) (**Figure 3,**
271 **Supplementary Table 2**).
272 *Gene Ontology terms associated with differentially methylated region-containing genes*

271 **Supplementary Table 2**).

272 *Gene Ontology terms assoc*

274 275 DMRs were annotated usin

276 containing genes across sex

277 Chipenrich was used to per

278 identify DMR-related GO t -- 1
273
274
275
277
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279 273 *Gene Ontology terms associated with differentially methylated region-containing genes*
274 DMRs were annotated using annotatr R Bioconductor package, and a summary of the overlocation
276 containing genes across sexes 275
276
277
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280
281
281 275 DMRs were annotated using annotatr R Bioconductor package, and a summary of the overlap in DMR-

276 containing genes across sexes, tissues, and exposures can be found in **Supplementary Figure 1**.

277 Chipenrich was u containing genes across sexes, tissues, and exposures can be found in **Supplementary Figure 1**.

277 Chipenrich was used to perform geneset enrichment tests and Gene Ontology (GO) Resource was

278 identify DMR-related GO 277 Chipenrich was used to perform geneset enrichment tests and Gene Ontology (GO) Resource was used to

278 identify DMR-related GO terms. The number of DMR-containing genes associated with each GO result

279 from both P

278 identify DMR-related GO terms. The number of DMR-containing genes associated with each GO result
279 from both Pb- and DEHP-exposed samples are summarized in **Supplementary Table 3**.
280 Within Pb-exposed tissues, cort

279 from both Pb- and DEHP-exposed samples are summarized in **Supplementary Table 3**.

280 Within Pb-exposed tissues, cortex had the greatest number of Gene Ontology Biological

281 (GOBP)-related DMR-containing genes in b Within Pb-exposed tissues, cortex had the greatest number of Gene Ontology Biological Pathway

281 (GOBP)-related DMR-containing genes in both males (85) and females (94). DMR-associated GC

282 female cortex were dominate 281 (GOBP)-related DMR-containing genes in both males (85) and females (94). DMR-associated GOBPs in

282 female cortex were dominated by metabolic processes (35 out of 94 genes), whereas male cortex

283 contained an abun

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282 female cortex were dominated by metabolic processes (35 out of 94 genes), whereas male cortex

283 contained an abundance of DMR-containing genes related to gene expression regulation (e.g., DN

284 methylation or deme contained an abundance of DMR-containing genes related to gene expression regulation (e.g., DNA

284 methylation or demethylation and miRNA gene silencing) (16 out of 85). The most common biologic

285 process associated w 284 methylation or demethylation and miRNA gene silencing) (16 out of 85). The most common biological

285 process associated with Pb exposure was genomic imprinting (GO:0071514), which appeared in male

286 cortex, blood, 285 process associated with Pb exposure was genomic imprinting (GO:0071514), which appeared in male

286 cortex, blood, and liver, as well as female cortex. In total, DMRs were detected in 21 genes associated

287 in DEHP-286 cortex, blood, and liver, as well as female cortex. In total, DMRs were detected in 21 genes associated

287 with genomic imprinting in these tissues (**Figure 4**).

288 In DEHP-exposed samples, a greater number of DMRwith genomic imprinting in these tissues (**Figure 4**).

288 In DEHP-exposed samples, a greater number of DMI

290 terms compared to Pb-exposed, especially the female

291 various GOBPs, most notably those associated with c 288
289
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295
296 In DEHP-exposed samples, a greater number of DMR-containing genes were associated with various GO

290 terms compared to Pb-exposed, especially the female cortex, which contained 179 genes associated with

291 various GOBP 290 terms compared to Pb-exposed, especially the female cortex, which contained 179 genes associated with

291 various GOBPs, most notably those associated with development (e.g., organ development,

292 differentiation, a

291 various GOBPs, most notably those associated with development (e.g., organ development,

292 differentiation, and morphogenesis) (148 of 179). Male cortex contained far fewer GO term-

2012 DMRs compared to females (66

292 differentiation, and morphogenesis) (148 of 179). Male cortex contained far fewer GO term-associated

293 DMRs compared to females (66 compared to 179), and there was an abundance of genes associated wit

294 gene exp

293 DMRs compared to females (66 compared to 179), and there was an abundance of genes associated with

294 gene expression regulation (10) and cellular organization (20). As with Pb-exposed tissues, the only GO

295 term 294 gene expression regulation (10) and cellular organization (20). As with Pb-exposed tissues, the only GO

295 term common to more than one tissue-sex combination among DEHP samples was genomic imprinting,

296 which was term common to more than one tissue-sex combination among DEHP samples was genomic imprinting,

296 which was associated with DMRs in 9 genes across male blood and cortex (**Figure 5**).

297 DNA methylation changes at impri which was associated with DMRs in 9 genes across male blood and cortex (**Figure 5**).

297

298 DNA methylation changes at imprinted loci

299 The appearance of imprinted genes in both exposure models during pathway analysi 297
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304 298 *DNA methylation changes at imprinted loci*
299 The appearance of imprinted genes in both
300 was motivation to take a closer look at the e
301 tissue types, across both sexes and exposure
302 **(Supplementary Figures 2** 299 The appearance of imprinted genes in both exposure models during pathway analysis (**Figures 4 and 5**)

300 was motivation to take a closer look at the effects of Pb and DEHP exposure on imprinted genes. All

301 tissue 300 was motivation to take a closer look at the effects of Pb and DEHP exposure on imprinted genes. All
301 tissue types, across both sexes and exposures had detectable changes in DNAm within imprinted gene
302 (Supplement 301 tissue types, across both sexes and exposures had detectable changes in DNAm within imprinted genes
302 (Supplementary Figures 2-5). A reference list of imprinted genes used in this analysis can be found in
303 **Supple** 302 (**Supplementary Figures 2-5**). A reference list of imprinted genes used in this analysis can be found in
303 **Supplemental Table 4**, and genes that did not contain a DMR in any tissue were omitted from the final
304 fi **Supplemental Table 4**, and genes that did not contain a DMR in any tissue were omitted from the final

304 figure. Cortex had the greatest number of DMRs as well as the greatest magnitude of methylation change

305 in ass 304 figure. Cortex had the greatest number of DMRs as well as the greatest magnitude of methylation changes
305 in assessed imprinted genes. 73 Pb-associated DMRs were detected in cortex at imprinted genes (46 in
306 males 305 in assessed imprinted genes. 73 Pb-associated DMRs were detected in cortex at imprinted genes (46 in males and 27 in females with magnitude changes of 5.03-23.77%) and 67 were detected in DEHP-

207 exposed cortex (37 306 males and 27 in females with magnitude changes of 5.03-23.77%) and 67 were detected in DEHP-

307 exposed cortex (37 in males and 30 in females with magnitude changes of 5.2-24.9%). 36 Pb-assoc

308 DMRs were detected 307 exposed cortex (37 in males and 30 in females with magnitude changes of 5.2-24.9%). 36 Pb-associated
308 DMRs were detected in blood at imprinted genes (16 in males and 20 in females with magnitude changes
309 of 5.04-308 DMRs were detected in blood at imprinted genes (16 in males and 20 in females with magnitude changes
309 of 5.04-20.1%) and 55 were detected in DEHP-exposed blood (32 in males and 23 in females with
310 magnitude chang 309 of 5.04-20.1%) and 55 were detected in DEHP-exposed blood (32 in males and 23 in females with
310 magnitude changes of 5.4-28.4%). Liver contained fewer changes in DNAm at imprinted genes,
311 compared to blood and cor 310 magnitude changes of 5.4-28.4%). Liver contained fewer changes in DNAm at imprinted genes,
311 compared to blood and cortex, for each sex-exposure combination, with 10 DMRs in Pb-exposed
312 in males and 1 in females w 311 compared to blood and cortex, for each sex-exposure combination, with 10 DMRs in Pb-exposed liver (9 in males and 1 in females with magnitude changes of 6.8-19.4%) and 11 DMRs in DEHP-exposed liver (3 in males and 8 in 312 in males and 1 in females with magnitude changes of 6.8-19.4%) and 11 DMRs in DEHP-exposed liver (3
313 in males and 8 in females with magnitude changes of 8.8-16.3%). Blood from Pb-exposed females largely
314 containe 313 in males and 8 in females with magnitude changes of 8.8-16.3%). Blood from Pb-exposed females largely
314 contained hypermethylated sites at imprinted genes (15/20 DMRs), while cortex from the same animals
315 was lar 314 contained hypermethylated sites at imprinted genes ($15/20$ DMRs), while cortex from the same animals was largely hypomethylated in the same gene class ($20/27$ DMRs). A similar pattern was seen in DEHP. was largely hypomethylated in the same gene class (20/27 DMRs). A similar pattern was seen in DEHP-
315 was largely hypomethylated in the same gene class (20/27 DMRs). A similar pattern was seen in DEHP-

316 male tissues, with the bulk of detectable changes found in the blood and cortex, with the former being
317 largely hypermethylated (29/32 DMRs in blood) and the latter hypomethylated 23/37 DMRs in cortex)
318 **(Supple** 317 largely hypermethylated (29/32 DMRs in blood) and the latter hypomethylated 23/37 DMRs in cortex)

318 (Supplementary Table 5).

319 Two imprinted genes, *Gnas* and *Grb10*, contained a notable number of exposure asso 318 **(Supplementary Table 5).**
319 Two imprinted genes, *Gnas*
320 complete overview of these
321 exposed samples, 60% and 7
322 respectively. In Pb-exposed
323 females (1/1) and hypometh
324 (2/2). Among Pb-exposed c Two imprinted genes, *Gnas* and *Grb10*, contained a notable number of exposure associated DMRs. A

320 complete overview of these DMRs is summarized in **Figure 6** and **Supplementary Table 6**. Among F

821 exposed samples, 320 complete overview of these DMRs is summarized in **Figure 6** and **Supplementary Table 6**. Among Pb-

321 exposed samples, 60% and 75% of DMRs in the *Gnas* locus were hypomethylated in males and females,

322 respective 321 exposed samples, 60% and 75% of DMRs in the *Gnas* locus were hypomethylated in males and females,
322 respectively. In Pb-exposed blood, DMRs within the *Gnas* locus were entirely hypermethylated in
323 females (1/1) 322 respectively. In Pb-exposed blood, DMRs within the *Gnas* locus were entirely hypermethylated in females (1/1) and hypomethylated in males (3/3). In Pb-exposed liver, *Gnas* DMRs were hypermethylated in $(2/2)$. Among

323 females (1/1) and hypomethylated in males (3/3). In Pb-exposed liver, *Gnas* DMRs were hypermethylated (2/2). Among Pb-exposed cortex, DMRs within the *Grb10* locus were largely hypermethylated in males (66%) and hypom

324 (2/2). Among Pb-exposed cortex, DMRs within the *Grb10* locus were largely hypermethylated in males (66%) and hypomethylated in females (66%). A similar pattern presented in Pb-exposed blood, wherein the entirety of

325 (66%) and hypomethylated in females (66%). A similar pattern presented in Pb-exposed blood, wherein
326 the entirety of *Grb10* DMRs in males were hypermethylated (2/2), whereas those in females were
327 hypomethylate

326 the entirety of *Grb10* DMRs in males were hypermethylated (2/2), whereas those in females were
327 hypomethylated (1/1). Male liver contained only hypermethylated sites (2/2) within the *Grb10* loc
328 DEHP exposure 327 hypomethylated (1/1). Male liver contained only hypermethylated sites (2/2) within the *Grb10* locus.
328 DEHP exposure was associated with more hypomethylation at the *Gnas* locus in male cortex (80%) the in females (328
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336 329 DEHP exposure was associated with more hypomethylation at the *Gnas* locus in male cortex (80%) than
330 in females (50%). In blood, DEHP exposure associated with more hypomethylation in females (75%) but
331 DMRs asso 330 in females (50%). In blood, DEHP exposure associated with more hypomethylation in females (75%) but
331 DMRs associated with this exposure in male blood were entirely hypermethylated (3/3). Regarding
322 $Grb10$, 2/3 D

331 DMRs associated with this exposure in male blood were entirely hypermethylated (3/3). Regarding
332 *Grb10*, 2/3 DMRs identified in male cortex were hypomethylated whereas 2/2 identified in male blowere hypermethylate 332 *Grb10*, 2/3 DMRs identified in male cortex were hypomethylated whereas 2/2 identified in male blood
333 were hypermethylated. One hypermethylated DMR was detected in *Grb10* in DEHP-exposed female
334 liver.
335 *Expo* were hypermethylated. One hypermethylated DMR was detected in *Grb10* in DEHP-exposed female

334 liver.

335 *Exposure-associated changes in imprinting control regions*

337 Imprinted genes are regulated in part through i 334 liver.

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336 *Expos*

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340 imprii Exposure-associated changes in imprinting control regions
337 Imprinted genes are regulated in part through imprinting co
338 whose methylation is set up in the germline and that regulat
339 imprinted gene clusters.³⁶ Ch

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342 337 Imprinted genes are regulated in part through imprinting control regions (ICRs), which are elements
338 whose methylation is set up in the germline and that regulate gene expression and subsequent function
339 imprint 338 whose methylation is set up in the germline and that regulate gene expression and subsequent functions of
339 imprinted gene clusters.³⁶ Changes in the DNAm status of these regions can impact the expression of
340 i imprinted gene clusters.³⁶ Changes in the DNAm status of these regions can impact the expression of

339 imprinted gene clusters.³⁶ Changes in the DNAm status of these regions can impact the expression of imprinted and non-imprinted genes within a given cluster, thus magnifying the regulatory effects of would otherwise 340 imprinted and non-imprinted genes within a given cluster, thus magnifying the regulatory effects of what would otherwise be a single-gene effect.³⁷ *Gnas* contains two ICRs, the *Gnas* ICR and the *Nespas* ICR, whil would otherwise be a single-gene effect.³⁷ *Gnas* contains two ICRs, the *Gnas* ICR and the *Nespas* ICR,

342 while *Grb10* contains one ICR.^{36,38} The current analysis identified multiple DMRs within the ICRs of

343 while *Grb10* contains one ICR.^{36,38} The current analysis identified multiple DMRs within the ICRs of

343 both *Gnas* (7 in ICR *Gnas* and 8 in ICR *Nespas*) and *Grb10* (17 in the *Grb10* ICR) across exposure are

344

343 both *Gnas* (7 in ICR *Gnas* and 8 in ICR *Nespas*) and *Grb10* (17 in the *Grb10* ICR) across exposure and
344 tissue types (**Figure 7**). A binomial test was conducted to assess whether exposure-associated DMRs
345 oc 344 tissue types (**Figure 7**). A binomial test was conducted to assess whether exposure-associated DMRs
345 occurred in these ICRs to a greater degree than would have been expected by random change. Both th
346 *Gnas* and

345 occurred in these ICRs to a greater degree than would have been expected by random change. Both the
346 *Gnas* and *Grb10* ICRs contained more DMRs than would have been expected by chance in multiple sex
347 exposure-*Gnas* and *Grb10* ICRs contained more DMRs than would have been expected by chance in multiple sex-

347 exposure-tissue combinations. A summary of these findings can be found in **Supplementary Table 6**.

348 Pb exposure

exposure-tissue combinations. A summary of these findings can be found in **Supplementary Table 6**.

348 Pb exposure was associated with relatively limited changes in DNAm in *Gnas* ICRs when compared to *Grb10*. In the *Ne* 348
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356 349 Pb exposure was associated with relatively limited changes in DNAm in *Gnas* ICRs when compared to *Grb10*. In the *Nespas* ICR, Pb exposure was associated with hypermethylation in female cortex (1/1 DMR) and a mix of 350 *Grb10*. In the *Nespas* ICR, Pb exposure was associated with hypermethylation in female cortex (1/1 DMR) and a mix of hyper- (1/2 DMRs) and hypomethylation (1/2 DMRs) in male cortex. In the *Gna* ICR, Pb exposure was 351 DMR) and a mix of hyper- (1/2 DMRs) and hypomethylation (1/2 DMRs) in male cortex. In the *Gnas*
352 ICR, Pb exposure was associated only with hypermethylation in male liver (1/1 DMR) (**Figure 7A** and
353 **Supplementar**

352 ICR, Pb exposure was associated only with hypermethylation in male liver (1/1 DMR) (**Figure 7A** and
353 **Supplementary Table 7**). In the *Grb10* ICR, Pb exposure was associated again with an equal amount o
354 hyper- (**Supplementary Table 7**). In the *Grb10* ICR, Pb exposure was associated again with an equal amount of

1954 by the *Supplementhylated (3/6)* DMRs, in both male and female cortex. Pb exposure was entirely

1955 associated 354 hyper- (3/6) and hypomethylated (3/6) DMRs, in both male and female cortex. Pb exposure was entirely
355 associated with hypermethylation in both male blood (2/2 DMRs) and liver (2/2 DMRs) but was
356 associated with 355 associated with hypermethylation in both male blood (2/2 DMRs) and liver (2/2 DMRs) but was
356 associated with hypomethylation in female blood (1/1 DMR) (**Figure 7B** and **Supplementary T**:
357 There were comparativel 356 associated with hypomethylation in female blood (1/1 DMR) (**Figure 7B** and **Supplementary Table 7**).
357 There were comparatively more changes in DNAm in the *Gnas* ICRs associated with DEHP exposure. In
359 male corte 358 There were comparatively more changes in DNAm in the *Gnas* ICRs associated with DEHP exposure. In male cortex there was again a mix of hyper- (1/2) and hypomethylated (1/2) DMRs in the *Nespas* ICR. Unlike Pb exposure

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364 359 male cortex there was again a mix of hyper- (1/2) and hypomethylated (1/2) DMRs in the *Nespas* ICR.
360 Unlike Pb exposure, DEHP was associated only with hypomethylated DMRs (2/2) in female cortex in t
361 *Nespas* IC 360 Unlike Pb exposure, DEHP was associated only with hypomethylated DMRs (2/2) in female cortex in the
361 *Nespas* ICR. In male blood there was 1 and 2 hypermethylated DEHP-associated DMRs within the
362 *Nespas* and *Gn* 361 *Nespas* ICR. In male blood there was 1 and 2 hypermethylated DEHP-associated DMRs within the
362 *Nespas* and *Gnas* ICRs, respectively. Female cortex and blood both contained a mix of hyper- (1/2)
363 hypomethylated 362 *Nespas* and *Gnas* ICRs, respectively. Female cortex and blood both contained a mix of hyper- (1/2) and
363 hypomethylated (1/2) DMRs in the *Gnas* ICR associated with DEHP exposure (**Figure 7A** and
364 **Supplementary**

363 hypomethylated (1/2) DMRs in the *Gnas* ICR associated with DEHP exposure (**Figure 7A** and **Supplementary Table 8**). Within the *Grb10* ICR, DEHP exposure was associated with a mix of **Supplementary Table 8**). Within t 364 **Supplementary Table 8**). Within the *Grb10* ICR, DEHP exposure was associated with a mix of hyper-

365 (1/3) and hypomethylated (2/3) DMRs in male cortex, hypermethylated (2/2) DMRs in male blood, and 1
366 hypermethylated DMR in female liver (**Figure 7B** and **Supplementary Table 8**).
367 **Discussion**
369 Toxicant expo

Toxicant exposures that occur during critical periods of development can have ramifications for health and well-being throughout the life-course.⁴⁵ Perinatal Pb and DEHP exposures have been linked to

- aberrant brain development and metabolic function, respectively, at environmentally relevant doses.^{46,47}
- 366 hypermethylated DMR in female liver (**Figure 7B** and **Supplementary Table 8**).
367 **Discussion**
369 Toxicant exposures that occur during critical periods of development can have ran
370 and well-being throughout the li 367
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374 368 **Discussion**
369 Toxicant ex
370 and well-be
371 aberrant bra
372 With regard
373 been associa
374 toxicant-ind 369 Toxicant exposures that occur during critical periods of development can have ramifications for health
370 and well-being throughout the life-course.⁴⁵ Perinatal Pb and DEHP exposures have been linked to
371 aberrant 370 and well-being throughout the life-course.⁴⁵ Perinatal Pb and DEHP exposures have been linked to
371 aberrant brain development and metabolic function, respectively, at environmentally relevant doses
372 With regard 372 With regard to epigenetic mechanisms governing gene expression, Pb and DEHP exposures have both
373 been associated with differential DNAm in human populations.^{48,49} Concurrently, it is unknown if
374 toxicant-induc
- been associated with differential DNAm in human populations.^{48,49}
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378 difficult to access tissues, as is being evaluated in the TaRGET II Consortium.²⁴

373 been associated with differential DNAm in human populations.^{48,49} Concurrently, it is unknown if
374 toxicant-induced changes in difficult-to-access tissues, such as brain and liver, are reflected in more
375 access 374 toxicant-induced changes in difficult-to-access tissues, such as brain and liver, are reflected in more easily
375 accessible (surrogate) tissues, such as blood. It is therefore pertinent to examine how two prominent
3 375 accessible (surrogate) tissues, such as blood. It is therefore pertinent to examine how two prominent
376 developmental exposures, Pb and DEHP, affect gene regulation by DNAm in these target and surrog
377 tissues in 376 developmental exposures, Pb and DEHP, affect gene regulation by DNAm in these target and surrogate
377 tissues in order to assess whether DNAm could be used as a potential biomarker of changes in more
378 difficult to 377 tissues in order to assess whether DNAm could be used as a potential biomarker of changes in more
378 difficult to access tissues, as is being evaluated in the TaRGET II Consortium.²⁴
379 *Pb and DEHP Exposures are*

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- *Pb and DEHP Exposures are Associated with Sex, Tissue, and Exposure-Specific General Changes in*

380 *DNA Methylation*. Overall, Pb and DEHP exposures resulted in similar number of DMRs between the

381 sexes for each of **280** *DNA Methylation.* Overall, Pb and DEHP exposures resulted in similar number of DMRs between the sexes for each of the three tissues assessed (**Figure 2A**). The cortex contained the greatest number of DMRs for each e
- female brain following gestational Pb exposure as well as in male liver following DEHP exposure.^{50,51}
-
- 381 sexes for each of the three tissues assessed (**Figure 2A**). The cortex contained the greatest number of
382 DMRs for each exposure, followed by blood and liver. Between the sexes, females had more DMRs
383 across both 382 DMRs for each exposure, followed by blood and liver. Between the sexes, females had more DMRs
383 across both exposures in cortex and blood, while males had more DMRs in the liver (**Figure 2A**). The overall DNAm patte 383 across both exposures in cortex and blood, while males had more DMRs in the liver (**Figure 2A**). This
384 overall DNAm pattern is consistent with previous reports, which showed significant changes in DNAm
385 female br overall DNAm pattern is consistent with previous reports, which showed significant changes in DNAm in

385 female brain following gestational Pb exposure as well as in male liver following DEHP exposure.^{50,51}

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386 There was minimal overlap in DMRs between either cortex-blood (0.5-1.2% of total DMRs detected in
387 these tissues) or liver-blood (0.25-1.5% of total DMRs detected in these tissues) (**Figure 2B**). The large
388 degre 387 these tissues) or liver-blood (0.25-1.5% of total DMRs detected in these tissues) (**Figure 2B**). The largest
388 degree in DMR similarity between target-surrogate tissues was in DEHP-exposed female cortex and
390 blood 388 degree in DMR similarity between target-surrogate tissues was in DEHP-exposed female cortex and
389 blood (13 similar DMRs, 1.16% of all DMRs in those tissues), followed by DEHP-exposed male cor
390 and blood (10 simil blood (13 similar DMRs, 1.16% of all DMRs in those tissues), followed by DEHP-exposed male cortex
390 and blood (10 similar DMRs, 1.12% of all DMRs in those tissues). These findings suggest limited gener
391 overlap in DNA 390 and blood (10 similar DMRs, 1.12% of all DMRs in those tissues). These findings suggest limited general
391 overlap in DNAm changes across surrogate and target tissues when stratified by sex and exposure.
392 When the 391 overlap in DNAm changes across surrogate and target tissues when stratified by sex and exposure.

392 When the similarity of DMR signatures between the sexes was assessed for Pb and DEHP exposure

394 greatest number 393
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400 393 When the similarity of DMR signatures between the sexes was assessed for Pb and DEHP exposures, the greatest number of shared DMRs was seen in the cortex, followed by blood, with no common DMRs in the liver (**Figure 2**

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- specific effects in toxicoepigenetic studies. $52,53$
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- 394 greatest number of shared DMRs was seen in the cortex, followed by blood, with no common DMRs in
395 the liver (**Figure 2C**). The number of DMRs in common between the sexes did not exceed 2% of the tot
396 DMRs detect
- 395 the liver (**Figure 2C**). The number of DMRs in common between the sexes did not exceed 2% of the total
396 DMRs detected in any tissue-exposure combination. These findings highlight the need to evaluate sex-
397 speci 396 DMRs detected in any tissue-exposure combination. These findings highlight the need to evaluate sex-

397 specific effects in toxicoepigenetic studies.^{52,53} A greater degree of DMR similarity was seen between

398 e 398 exposure types, with 1-7% of total DMRs appearing in both Pb and DEHP-exposed tissues, depending on the sex and tissue (**Figure 2D**). General trends in DMR directionality were not conserved across tissue types, adding 398 exposure types, with 1-7% of total DMRs appearing in both Pb and DEHP-exposed tissues, depending on
399 the sex and tissue (**Figure 2D**). General trends in DMR directionality were not conserved across tissue
400 types 399 the sex and tissue (**Figure 2D**). General trends in DMR directionality were not conserved across tissue
400 types, adding complexity to comparisons of changes in DNAm patterns between target and surrogate
401 tissues (types, adding complexity to comparisons of changes in DNAm patterns between target and surrogate

401 tissues (**Figure 2E**). As expected, many DMRs were located in CpG islands, areas of dynamic DNAn

402 directed gene expr 401 tissues (**Figure 2E**). As expected, many DMRs were located in CpG islands, areas of dynamic DNAm-
402 directed gene expression regulation.⁵⁴ Gene promoters and exons also contained more DMRs than wou
403 have been pr directed gene expression regulation.⁵⁴ Gene promoters and exons also contained more DMRs than would
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- 402 directed gene expression regulation.³⁴ Gene promoters and exons also contained more DMRs than would
403 have been predicted by chance (**Figure 3**).
404 *Exposure-Associated DMRs Occur to a Notable Degree in Imprinte* have been predicted by chance (**Figure 3**).

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405 Exposure-Associated DMRs Occur to a No.

406 associated with DMR-containing genes ide

407 tissues in both sexes and exposure types (**F**

408 regard to early growth and
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- nature of expression may confer particular susceptibility to the impacts of environmental exposures.^{55,56}
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412 *Exposure-Associated DMRs Occur to a Notable Degree in Imprinted Genes.* An analysis of GO terms

406 associated with DMR-containing genes identified genomic imprinting as a common category across m

407 tissues in both se 410
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- 410 Early disruption of imprinted gene expression and function can result in developmental disorders (e.g., pseudohypoparathyroidism type 1B and Silver-Russell syndrome, for which perturbations in gene expression regulati 411 pseudohypoparathyroidism type 1B and Silver-Russell syndrome, for which perturbations in gene
412 expression regulation of *Gnas* and *Grb10*, respectively, have been implicated.^{57,58} Additionally, ch
413 in the DNA expression regulation of *Gnas* and *Grb10*, respectively, have been implicated.^{57,58}
- 406 associated with DMR-containing genes identified genomic imprinting as a common category across most
407 tissues in both sexes and exposure types (**Figures 4 and 5**). Imprinted genes are an important class with
408 reg 407 tissues in both sexes and exposure types (**Figures 4 and 5**). Imprinted genes are an important class with regard to early growth and development, and their epigenetically-controlled mono-allelic parent of origin natur 408 regard to early growth and development, and their epigenetically-controlled mono-allelic parent of origin
409 nature of expression may confer particular susceptibility to the impacts of environmental exposures.^{55,56} 412 expression regulation of *Gnas* and *Grb10*, respectively, have been implicated.^{57,38} Additionally, changes in the DNAm status of several imprinted genes have been associated with chronic conditions such as 413 in the DNAm status of several imprinted genes have been associated with chronic conditions such as

- diabetes, cardiovascular disease, and cancer.⁵⁹⁻⁶¹ The DNAm and hydroxymethylation status of imprinted 414 diabetes, cardiovascular disease, and cancer.⁵⁹⁻⁶¹ The DNAm and hydroxymethylation status of imprinted
415 genes is particularly susceptible to environmental exposures during early development, including Pb and
416
- quares is particularly susceptible to environmental exposures during early development, including Pb and

416 DEHP.^{62–64} Epidemiological studies have linked early life Pb exposure to altered methylation in imprinted

41 $DEHP.^{62–64}$
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- 416 DEHP.⁶²⁻⁶⁴ Epidemiological studies have linked early life Pb exposure to altered methylation in imprinted
417 genes including insulin-like growth factor 2 (*IGF2*), which is involved in some cases of Beckwith-
418 W quare including insulin-like growth factor 2 (*IGF2*), which is involved in some cases of Beckwith-

418 Wiedemann Syndrome and Silver-Russell Syndrome and maternally expressed gene 3 (*MEG3*), wh

419 implicated in Temple Wiedemann Syndrome and Silver-Russell Syndrome and maternally expressed gene 3 (*MEG3*), which is

419 implicated in Temple syndrome and Kagami-Ogata syndrome.).^{65,66}

420 *Imprinting Control Regions Contain Exposure- an* implicated in Temple syndrome and Kagami-Ogata syndrome.).^{65,66}
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427 consequences for a cluster of imprinted genes.⁶⁷
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- *Imprinting Control Regions Contain Exposure- and Tissue-Specific Changes in DNA Methylation*. ICRs

422 are environmentally sensitive regulatory regions, and changes to their DNAm status can have

423 consequences for a c 422 are environmentally sensitive regulatory regions, and changes to their DNAm status can have
423 consequences for a cluster of imprinted genes.⁶⁷ The ICRs of both *Gnas* and *Grb10* contained
424 Pb- and DEHP-associa 223 consequences for a cluster of imprinted genes.⁶⁷ The ICRs of both *Gnas* and *Grb10* contained numerous
224 Pb- and DEHP-associated DMRs, with *Gnas* ICR DMRs appearing largely in the cortex and to be more
225 preval 424 Pb- and DEHP-associated DMRs, with *Gnas* ICR DMRs appearing largely in the cortex and to be more
425 prevalent with DEHP exposure, while the *Grb10* ICR contained about twice as many Pb-associated
426 DMRs than DEHP a quared the Arb 10 DEHP exposure, while the *Grb10* ICR contained about twice as many Pb-associated

426 DMRs than DEHP and with much more even distribution across the studied tissues.

427 The *Grb10* ICR contained DMRs a 426 DMRs than DEHP and with much more even distribution across the studied tissues.
427 The *Grb10* ICR contained DMRs across all three tissues examined, with a specific L
428 exposed male liver and blood. There was an ad
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- 428 exposed male liver and blood. There was an additional DMR in common in DEHP-exposed male cortex
429 and blood, but they differed in directionality (cortex = hypomethylated, blood = hypermethylated). The
430 current st
- 427 The *Grb10* ICR contained DMRs across all three tissues examined, with a specific DMR replicated in Pb-
428 exposed male liver and blood. There was an additional DMR in common in DEHP-exposed male cortex
429 and blood
- ICR, with a previous report highlighting the effects on hydroxymethylation, 64 though many more exist
- pertaining to changes throughout the gene.^{68,69}
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- 429 and blood, but they differed in directionality (cortex = hypomethylated, blood = hypermethylated). The
430 current study is one the few reports that examine the effects of environmental exposures on the *Grb10*
431 IC 430 current study is one the few reports that examine the effects of environmental exposures on the *Grb10* ICR, with a previous report highlighting the effects on hydroxymethylation,⁶⁴ though many more exist pertaining 431 ICR, with a previous report highlighting the effects on hydroxymethylation,⁶⁴ though many more exist
432 pertaining to changes throughout the gene.^{68,69} Much of the published work is restricted to germ cells, a
43 432 pertaining to changes throughout the gene.^{88,69} Much of the published work is restricted to germ cells, and
433 so additional work is needed to assess whether *Grb10* regulation and function are impacted by the
434 433 so additional work is needed to assess whether *Grb10* regulation and function are impacted by the
434 environment in the soma.
435 *Differential Methylation of Gnas and Grb10 Occurred in Gene Expression Regulatory Re* 434 environment in the soma.

435 *Differential Methylation of*

436 encodes for the G-protein

437 generation⁷⁰, and its impri

438 neural tube defects, and h₁

440 highly tissue-specific in m

440 highly tissue-spec *Differential Methylation of Gnas and Grb10 Occurred in Gene Expression Regulatory Regions. Gnas* encodes for the G-protein alpha-subunit protein, which contributes to signal transduction via cAMP generation⁷⁰, and its i 436 encodes for the G-protein alpha-subunit protein, which contributes to signal transduction via cAMP
437 generation⁷⁰, and its imprinting dysregulation has been associated with increased insulin sensitivity,
438 gives generation⁷⁰, and its imprinting dysregulation has been associated with increased insulin sensitivity, queneration⁷⁰, and its imprinting dysregulation has been associated with increased insulin sensitivity,

438 eural tube defects, and hypothyroidism.^{71,72} The imprinted expression of *Gnas* is complex, as this g

439 g 438 neural tube defects, and hypothyroidism.^{$11,12$} The imprinted expression of *Gnas* is complex, as this gene
439 gives rise to several maternal- and paternal-specific gene products, and these patterns of expression a 439 gives rise to several maternal- and paternal-specific gene products, and these patterns of expression are highly tissue-specific in mice and humans.⁷³⁻⁷⁵ In this work, *Gnas* contained a mix of hyper- and hypomethyl highly tissue-specific in mice and humans.^{73–75} In this work, *Gnas* contained a mix of hyper- and
441 hypomethylated DMRs in the cortex, under both exposure conditions (**Figure 6**), making the prec
6 of the observed su 441 hypomethylated DMRs in the cortex, under both exposure conditions (**Figure 6**), making the prediction
442 of the observed sustained DNAm effects at 5 months difficult to ascertain. However, given the
443 importance of 442 of the observed sustained DNAm effects at 5 months difficult to ascertain. However, given the
443 importance of maintained imprinted expression of this locus and its various gene products in the
444 continued evaluati 443 importance of maintained imprinted expression of this locus and its various gene products in the brain,
444 continued evaluation of the effects of exposure-induced changes in DNAm at this locus would help
445 elucidat 444 continued evaluation of the effects of exposure-induced changes in DNAm at this locus would help
445 elucidate the functional impacts on gene product expression and subsequent physiological effects.
446 Changes in DNA 445 elucidate the functional impacts on gene product expression and subsequent physiological effects.
446 Changes in DNAm within *Gnas* were much more uniform in blood and liver, where biallelic expreconsidered to be the Changes in DNAm within *Gnas* were much more uniform in blood and liver, where biallelic expression is

447 considered to be the norm in adult mice.⁷⁰ Distinct differences in *Gnas* DMR direction appeared between

448 th considered to be the norm in adult mice.⁷⁰ Distinct differences in *Gnas* DMR direction appeared between 447 considered to be the norm in adult mice.⁷⁰ Distinct differences in *Gnas* DMR direction appeared between
448 the sexes in this study (**Figure 6**). In Pb-exposed blood, *Gnas* DMRs were entirely hypomethylated in
449 448 the sexes in this study (**Figure 6**). In Pb-exposed blood, *Gnas* DMRs were entirely hypomethylated in males and hypermethylated in females. Within DEHP-exposed blood, *Gnas* DMRs were entirely hypermethylated in males males and hypermethylated in females. Within DEHP-exposed blood, *Gnas* DMRs were entirely
450 hypermethylated in males and a mix of hyper- and hypomethylated in females (**Figure 6**). As this
451 found hypomethylation in
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- 450 hypermethylated in males and a mix of hyper- and hypomethylated in females (**Figure 6**). As this work
451 found hypomethylation in *Gnas* promoters of DEHP-exposed female cortex and blood, it would be
452 pertinent to 451 found hypomethylation in *Gnas* promoters of DEHP-exposed female cortex and blood, it would be
452 pertinent to expand this work to additional tissues such as the thyroid to ascertain whether this
453 relationship is c 452 pertinent to expand this work to additional tissues such as the thyroid to ascertain whether this
453 relationship is consistent in an organ known to be significantly impacted by developmental cha
454 *Gnas* DNAm stat 453 relationship is consistent in an organ known to be significantly impacted by developmental changes in
454 *Grb10* encodes for an insulin receptor-binding protein involved in growth and insulin response and is
455 *Grb* 454 *Gnas* DNAm status.
455 *Grb10* encodes for an
456 imprinted in a tissue-
457 changes in *Grb10* ex
459 *Grb10* in DEHP-exp
460 appears to be cell-typ
- 455 *Grb10* encodes for an insulin receptor-binding protein involved in growth and insulin response and is imprinted in a tissue- and cell-type specific manner.^{76,77} This is especially true during development, as change imprinted in a tissue- and cell-type specific manner.^{76,77} 456 imprinted in a tissue- and cell-type specific manner.^{'6,17} This is especially true during development, as changes in $Grb10$ expression across time are tissue-specific. For example, in the brain, $Grb10$ imprintitions
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- 457 changes in $Grb10$ expression across time are tissue-specific. For example, in the brain, $Grb10$ imprinting
458 status is cell-type specific during development until adulthood.⁷⁸ There were several DMRs detected in
4 status is cell-type specific during development until adulthood.⁷⁸
- 458 status is cell-type specific during development until adulthood.⁷⁸ There were several DMRs detected in $Grb10$ in DEHP-exposed male cortex, as well as Pb-exposed male and female cortex. $Grb10$ methylatic appears to b
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- 459 *Grb10* in DEHP-exposed male cortex, as well as Pb-exposed male and female cortex. *Grb10* methylation appears to be cell-type specific during early brain development, with paternal expression in cortical neurons and 460 appears to be cell-type specific during early brain development, with paternal expression in cortical neurons and maternal expression in glial cells.⁷⁷ While this study was unable to assess cell-type specific specif neurons and maternal expression in glial cells.⁷⁷ neurons and maternal expression in glial cells.⁷⁷ While this study was unable to assess cell-type specific
1

462 changes in DNAm within the cortex, future single-cell analyses could help determine whether exposure-
463 associated DMRs are specific to certain cellular populations. $Grb10$ expression also changes significant
464 in 463 associated DMRs are specific to certain cellular populations. *Grb10* expression also changes significantly
464 in the liver during development, as maternal expression is high during fetal development, but nearly all
 464 in the liver during development, as maternal expression is high during fetal development, but nearly all
465 $Grb10$ expression is silenced in the liver in adulthood.^{79,80} Many of the DMRs seen in $Grb10$ in the live
 Grb10 expression is silenced in the liver in adulthood.^{79,80} 465 *Grb10* expression is silenced in the liver in adulthood.^{79,80} Many of the DMRs seen in *Grb10* in the liver were hypermethylated, suggesting these exposures may not result in the reactivation of this gene in adulth 466 were hypermethylated, suggesting these exposures may not result in the reactivation of this gene in
467 adulthood, but, alternatively, may reinforce its suppression through supplemental methylation. Whe
468 this trend 467 adulthood, but, alternatively, may reinforce its suppression through supplemental methylation. Whether
468 this trend was present during early development, when imprinted expression is the norm and whether tha
469 had 468 this trend was present during early development, when imprinted expression is the norm and whether that
469 had any deleterious effects on liver development, remains to be seen. Pb exposure, on the other hand, was
470

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- 469 had any deleterious effects on liver development, remains to be seen. Pb exposure, on the other hand, was
470 associated with hypomethylation of $Grb10$ in female blood, another tissue in which $Grb10$ is thought to
471 470 associated with hypomethylation of *Grb10* in female blood, another tissue in which *Grb10* is thought to be maternally expressed during early development and completely repressed during adulthood,⁸⁰ meanin that exp be maternally expressed during early development and completely repressed during adulthood,⁸⁰ meaning
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- specific expression from an alternate promoter. $\frac{7}{7}$
- 471 be maternally expressed during early development and completely repressed during adulthood,⁸⁰ meaning
472 that exposure may be related to reactivation of this gene during an inappropriate time point. Future
473 eval 472 that exposure may be related to reactivation of this gene during an inappropriate time point. Future
473 evaluation of the impact of $Grb10$ expression in blood during adulthood would contribute to our
474 understandin evaluation of the impact of *Grb10* expression in blood during adulthood would contribute to our

474 understanding of the potential functional impact of this change in methylation. *Grb10* is initially

475 expressed from understanding of the potential functional impact of this change in methylation. *Grb10* is initially

475 expressed from the maternal allele in somatic lineages and exclusively in neurons, switches to pa

476 specific expr 475 expressed from the maternal allele in somatic lineages and exclusively in neurons, switches to paternal-
476 specific expression from an alternate promoter.⁷⁷
477 Gnas and Grb10 Provide Evidence of DNA Methylation S
- 477 *Gnas and Grb10 Provide Evidence of DNA Methylation Signatures in Target-Surrogate Tissue Pairs.* The
478 ICRs of both *Gnas* and *Grb10* displayed some changes in DNAm that were replicated in both target and
479 surro
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- 476
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- 178 ICRs of both *Gnas* and *Grb10* displayed some changes in DNAm that were replicated in both target and

179 surrogate tissues, suggesting these regulatory regions may be of significance when attempting to identify

179
-
- 479 surrogate tissues, suggesting these regulatory regions may be of significance when attempting to identify
480 DNAm-related biomarkers of exposure (**Figure 7**). Among Pb-exposed samples, the *Grb10* ICR
481 contained hy DNAm-related biomarkers of exposure (**Figure 7**). Among Pb-exposed samples, the *Grb10* ICR

481 contained hypermethylated DMRs in male cortex, liver, and blood, suggesting that, for this exposer

482 *Grb10* ICR may be a 481 contained hypermethylated DMRs in male cortex, liver, and blood, suggesting that, for this exposure, the
482 Grb10 ICR may be a potential region to consider when exploring male-specific DNAm biomarkers of
483 exposure 482 *Grb10* ICR may be a potential region to consider when exploring male-specific DNAm biomarkers of exposure. Among DEHP-exposed samples, the *Gnas* ICR contained hyper- and hypomethylated DMR that were seen in female co 483 exposure. Among DEHP-exposed samples, the *Gnas* ICR contained hyper- and hypomethylated DMRs
484 that were seen in female cortex and blood, while the *Nespas* ICR was the location of hypermethylated
485 DMRs in male c 484 that were seen in female cortex and blood, while the *Nespas* ICR was the location of hypermethylated
485 DMRs in male cortex and blood. These findings suggest there may be ICR- and sex-specificity in term
486 of DNAm 2485 DMRs in male cortex and blood. These findings suggest there may be ICR- and sex-specificity in terms

2486 of DNAm biomarkers of DEHP exposure, and that they may be particularly applicable to the cortex and

2487 blo
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- 486 of DNAm biomarkers of DEHP exposure, and that they may be particularly applicable to the cortex and
487 blood. DEHP-associated hypermethylated DMRs were also replicated in male cortex and blood within the
488 *Grb10* 489 DEHP exposure.

490 **Limitations**

491 DNAm patterns v

492 type and therefore

493 induced changes

494 hydroxymethylati

494 which executs for 490 **Limitations**
491 DNAm patte
492 type and ther
494 hydroxymeth
495 which accour
496 two signature DNAm patterns vary across cell types within a given tissue.^{81,82}
- 487 blood. DEHP-associated hypermethylated DMRs were also replicated in male cortex and blood within the
488 *Grb10* ICR, suggesting this regulatory region may be an additional candidate as a DNAm biomarker for
489 **Limit** *Grb10* ICR, suggesting this regulatory region may be an additional candidate as a DNAm biomarker for

489 DEHP exposure.
 Limitations

291 DNAm patterns vary across cell types within a given tissue.^{81,82} This study wa
- type and therefore, changes in DNAm as the result of Pb or DEHP exposure may be due to exposure-

493 induced changes in cell type proportions.⁸³ Additionally, we were not able to evaluate changes in DN

494 hydroxymethy induced changes in cell type proportions.⁸³
- induced changes in cell type proportions.⁸³ Additionally, we were not able to evaluate changes in DNA
494 hydroxymethylation (5hmC) in these samples. This study was conducted using bisulfite conversion,
495 which account
- 494 hydroxymethylation (5hmC) in these samples. This study was conducted using bisulfite conversion,
495 which accounts for both 5mC and 5hmC, and the resulting data is unable to differentiate between the
496 two signature
- which accounts for both 5mC and 5hmC, and the resulting data is unable to differentiate between these

496 two signatures.⁶⁴. Imprinted genes are typically 50% methylated (accounting for mono-allelic expression

497 or r two signatures.⁶⁴. Imprinted genes are typically 50% methylated (accounting for mono-allelic expression
- 491 DNAm patterns vary across cell types within a given tissue.^{81,82} This study was unable to account for cell
492 type and therefore, changes in DNAm as the result of Pb or DEHP exposure may be due to exposure-
493 ind two signatures.⁶⁴. Imprinted genes are typically 50% methylated (accounting for mono-allelic expression

497 or repression), and this data represents DNAm averages for both alleles. Thus, any allele-specific changes

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- or repression), and this data represents DNAm averages for both alleles. Thus, any allele-specific changes

498 in DNAm associated with Pb or DEHP cannot be detected.

499 **Conclusion**

500 This study systematically evalua
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- in DNAm associated with Pb or DEHP cannot be detected.

499 **Conclusion**

500 This study systematically evaluated changes in DNAm for compared 501 5 months-of-age following developmental exposure to eithe

502 DNAm changes
- 499 **Conclusion**
500 This study s:
501 5 months-of-
502 DNAm chan
503 types, with lo
504 DEHP expos
505 changes in E 501 5 months-of-age following developmental exposure to either Pb or DEHP. Pb- and DEHP-specific
502 DNAm changes were observed via DMRs, with the greatest DMR similarity seen between exposur
503 types, with less overlap b 502 DNAm changes were observed via DMRs, with the greatest DMR similarity seen between exposure types, with less overlap between the sexes and tissues. Genomic imprinting was impacted by Pb and DEHP exposure, as determined types, with less overlap between the sexes and tissues. Genomic imprinting was impacted by Pb and
504 DEHP exposure, as determined by GO term analysis, and imprinted genes *Gnas* and *Grb10* indicated
505 changes in DNAm a
- This study systematically evaluated changes in DNAm for cortex, blood, and liver collected from mice at
501 5 months-of-age following developmental exposure to either Pb or DEHP. Pb- and DEHP-specific
502 DNAm changes were 504 DEHP exposure, as determined by GO term analysis, and imprinted genes *Gnas* and *Grb10* indicated
505 changes in DNAm at their respective ICRs. These results indicate that imprinted gene methylation can
506 dysregulat
- 505 changes in DNAm at their respective ICRs. These results indicate that imprinted gene methylation can be
506 dysregulated by developmental environmental exposures such as Pb and DEHP and that ICRs may be
1507 useful can
- 506 dysregulated by developmental environmental exposures such as Pb and DEHP and that ICRs may be useful candidates when exploring DNAm-based biomarkers of environmental exposures.
507 useful candidates when exploring DNA 507 useful candidates when exploring DNAm-based biomarkers of environmental exposures.

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510 Advanced Genomics
511 Center (M-LEEaD) w
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511 Center (M-LEEaD) which fa 510 Advanced Genomics Core, as well as the Michigan Lifestage Environmental Exposures and Disease
511 Center (M-LEEaD) which facilitated the generation and analysis of WGBS data.
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513 The authors report there are no competing interests to declare.

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516 Health Sciences (NII
517 Grant K01 (ES03204
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- 513 The authors report there are no competing interests to declare.

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515 This work

515 Health Sc

517 Grant K0

518 Exposure:

519 T32 (ES0

520 Grant R0**
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518 Exposures and Disease (M-LEEaD)
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520 Grant R01 (AG072 519 T32 (ES007062), Institutional Training Grant T32 (HD079342), and National Institute on Aging (NIA)
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521 **Data Sharing**
522 WGBS data will be uploaded to GEO. Additional data that support the fi 520 Grant R01 (AG072396).
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525 Work outlined

WGBS data will be uploaded to GEO. Additional data that support the findings of this study are available

523 from the corresponding author, DCD, upon reasonable request.

524 **Approval for Animal Use**

525 Work outlined i 523 from the corresponding author, DCD, upon reasonable request.
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525 **Work outlined in this manuscript was approved by the Universi
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530 525 **Approval for Animal Use**
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530 527 Work outlined in this manuscript was approved by the University of Michigan Institutional Animal Care
528 and Use Committee (IACUC) and conducted in accordance with the highest animal welfare standards.
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548 **Figure 1: Overview of experimental workflow.** F0 generation females (6-8 weeks of age) were exposed
543 to either 32ppm of Pb via drinking water or 5mg/kg-day of DEHP via food, beginning two weeks prior to
544 mating usin

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- to either 32ppm of Pb via drinking water or 5mg/kg-day of DEHP via food, beginning two weeks prior to
544 mating using virgin males (8-10 weeks of age). Exposure to Pb or DEHP or control continued through
545 gestation and 544 mating using virgin males (8-10 weeks of age). Exposure to Pb or DEHP or control continued through
545 gestation and weaning, when F1 mice were removed from the dams and placed on control water or cho
546 At 5 months o 546 At 5 months of age, F1 mice were sacrificed, and genomic DNA was extracted from blood, liver, and
547 cortex tissues. DNA was used to prepare libraries for Whole Genome Bisulfite Sequencing (WGBS).
548 Following initia Following initial data processing, Differentially Methylated Regions (DMRs) were called using

549 MethylSig and metilene.
 Figure 2: Summary of detected Differentially Methylated Regions. Differentially Methylated

(DMR
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- gestation and weaning, when F1 mice were removed from the dams and placed on control water or chow.

546 At 5 months of age, F1 mice were sacrificed, and genomic DNA was extracted from blood, liver, and

547 cortex tissues
-
- 547 cortex tissues. DNA was used to prepare libraries for Whole Genome Bisulfite Sequencing (WGBS).
548 Following initial data processing, Differentially Methylated Regions (DMRs) were called using
550 **Figure 2: Summary o** 549 MethylSig and metilene.
550 **Figure 2: Summary of d**
551 (DMRs) were categorized
552 group (Pb, DEHP, and co
553 categorized by sex and ex
554 were quantified and broke
555 summarized for each tiss **Figure 2: Summary of detected Differentially Methylated Regions.** Differentially Methylated Regions (DMRs) were categorized by tissue (blood, cortex, and liver), sex (F: female, M: male), and exposure group (Pb, DEHP, and 551 (DMRs) were categorized by tissue (blood, cortex, and liver), sex (F: female, M: male), and exposure group (Pb, DEHP, and control) (2A), and DMRs found in more than one tissue type were further categorized by sex and group (Pb, DEHP, and control) (2A), and DMRs found in more than one tissue type were further

553 categorized by sex and exposure (2B). DMRs shared by both sexes (2C) and by exposure group (

were quantified and broken dow
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categorized by sex and exposure (2B). DMRs shared by both sexes (2C) and by exposure group (2D)
554 were quantified and broken down by tissue type. Proportions of DMR directional changes were gener
555 summarized for each were quantified and broken down by tissue type. Proportions of DMR directional changes were generally

summarized for each tissue-sex-exposure combination, designated by DNA hyper (more methylated) or

hypo (less methylate summarized for each tissue-sex-exposure combination, designated by DNA hyper (more methylated) or
556 hypo (less methylated), in comparison to controls (2E).
557 **Figure 3: Genomic region of detected Differentially Methyla** hypo (less methylated), in comparison to controls (2E).
 Figure 3: Genomic region of detected Differentially

Regions (DMRs) were mapped to the mouse reference g

annotated as percentage of total DMRs (comparing cont

ex Figure 3: Genomic region of detected Differentially Methylated Regions. Differentially Methylated

558 Regions (DMRs) were mapped to the mouse reference genome (mm10) and their genomic region

559 annotated as percentage o

Example 1558 Regions (DMRs) were mapped to the mouse reference genome (mm10) and their genomic region

559 annotated as percentage of total DMRs (comparing control and exposed samples) for that sex and

560 exposure within annotated as percentage of total DMRs (comparing control and exposed samples) for that sex and

560 exposure within each tissue. This distribution was compared to what would be expected in a rando

561 distribution.

562 560 exposure within each tissue. This distribution was compared to what would be expected in a random
561 distribution.
562 **Figure 4: GO-terms associated with Differentially Methylated Region-containing genes among
564 ex** 561 distribution.
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565 submitted for
565 Cellular Con
567 **Figure 5: G**
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570 Figure 4: GO-terms associated with Differentially Methylated Region-containing genes among Pb-

sposed tissues. Differentially Methylated Region-containing genes found in Pb-exposed tissues were

submitted for Gene Ontolog **exposed tissues.** Differentially Methylated Region-containing genes found in Pb-exposed tissues were

submitted for Gene Ontology (GO) term analysis across three categories: Biological Process (GOBP),

Cellular Component

submitted for Gene Ontology (GO) term analysis across three categories: Biological Process (GOBP),

566 Cellular Component (GOCC), and Molecular Function (GOMF).

567 Figure 5: GO-terms associated with Differentially Methy Cellular Component (GOCC), and Molecular Function (GOMF).

567
 **Figure 5: GO-terms associated with Differentially Methylated

DEHP-exposed tissues.** Differentially Methylated Region-contai

tissues were submitted for Gene 568
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575 Figure 5: GO-terms associated with Differentially Methylated Region-containing genes among

DEHP-exposed tissues. Differentially Methylated Region-containing genes found in DEHP-exposed

fissues were submitted for Gene Ont **DEHP-exposed tissues.** Differentially Methylated Region-containing genes found in DEHP-exposed

570 tissues were submitted for Gene Ontology (GO) term analysis across three categories: Biological Proc

571 (GOBP), Cellula

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- 570 tissues were submitted for Gene Ontology (GO) term analysis across three categories: Biological Process
571 (GOBP), Cellular Component (GOCC), and Molecular Function (GOMF).
572 **Figure 6: Genomic location and directi** (GOBP), Cellular Component (GOCC), and Molecular Function (GOMF).

572 Figure 6: Genomic location and direction of Pb and DEHP-associated 1

Regions in the *Gnas* and *Grb10* loci. Differentially Methylated Regions (I

and 573
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575 Figure 6: Genomic location and direction of Pb and DEHP-associated Differentially Methylated

Regions in the *Gnas* and *Grb10* loci. Differentially Methylated Regions (DMRs) detected in the *Gna*.

and *Grb10* loci were c **Regions in the** *Gnas* **and** *Grb10* **loci.** Differentially Methylated Regions (DMRs) detected in the *Gnas* and *Grb10* loci were classified as to their genomic location within each gene. Percent change in methylation is den and *Grb10* loci were classified as to their genomic location within each gene. Percent change in

576 methylation is denoted by size and direction of methylation change by color (blue = hypermethy

577 DMRs among DEHP sam
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- 576 methylation is denoted by size and direction of methylation change by color (blue = hypermethylated
577 DMRs among DEHP samples, yellow = hypomethylation among DEHP samples, red = hypermethyla
578 among Pb samples, gr 577 DMRs among DEHP samples, yellow = hypomethylation among DEHP samples, red = hypermethylation
578 among Pb samples, green = among hypomethylation among Pb samples).
580 **Figure 7: Differentially Methylated Regions dete** 578 among Pb samples, green = among hypomethylation among Pb samples).
579 **Figure 7: Differentially Methylated Regions detected within** *Gnas* **and Regions (ICRs) among Pb and DEHP exposed tissues. (A) Differentiall overla** Figure 7: Differentially Methylated Regions detected within *Gnas* and *Grb10* Imprinting Control
581 Regions (ICRs) among Pb and DEHP exposed tissues. (A) Differentially Methylated Regions (DMR
582 overlap with *Gnas*. (B
- **Example 1581 Regions (ICRs) among Pb and DEHP exposed tissues.** (A) Differentially Methylated Regions (DMRs) overlap with *Gnas*. (B) DMRs overlap with *Grb10*. DMRs only represents the related genomic locations correspon 582 overlap with *Gnas*. (B) DMRs overlap with *Grb10*. DMRs only represents the related genomic locations
583 corresponding to the genomic coordinates of ICRs. The genomic coordinates of these DMRs can be foun
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585 583 corresponding to the genomic coordinates of ICRs. The genomic coordinates of these DMRs can be found
584 in Supplementary Table 4.
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584 in Supplementary Table 4.
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- 586 **References**

587 1Gillman N

588 *Medicine*

590 2Bernal AJ,

590 *Defects Refects Refects Refects Refects* 1 Sample 1 Gillman MW. Developmental origins of health and disease. The New England Journal of

588 Medicine 2005;353:1848.

589 2 Bernal AJ, Jirtle RL. Epigenomic disruption: the effects of early developmental exposures. 589 2 Bernal AJ, Jirtle RL. Epigen
590 Defects Research Part A: C
591 3 Bollati V, Baccarelli A. Env
592 4 Lyko F. The DNA methyltra
593 Reviews Genetics 2018;19
594 5 Siegfried Z, Simon I. DNA r
-
-
- Examples 2 Bernal AJ, Sirtle RL. Epigenomic disruption: the effects of early developmental exposures. Birth Defects Research Part A: Clinical and Molecular Teratology 2010;88:938–44.

591 3 Bollati V, Baccarelli A. Environ Begies Research Part A: Chincal and Molecular Feratology 2010;88:938–44.

591 Bellati V, Baccarelli A. Environmental epigenetics. Heredity 2010;105:105–12

4 Lyko F. The DNA methyltransferase family: a versatile toolkit fo 591 3 Bonati V, Baccarelli A. Environmental epigenetics. Heredity 2010,103.103–12.
592 4 Lyko F. The DNA methyltransferase family: a versatile toolkit for epigenetic rear
593 *Reviews Genetics* 2018;19:81–92.
594 5 Siegfri 592 4 Lyko F. The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. Nature
593 Reviews Genetics 2018;19:81–92.
594 5 Siegfried Z, Simon I. DNA methylation and gene expression. Wiley Interdiscipli 593 Reviews Genetics 2016,19:81–92.
594 Siegfried Z, Simon I. DNA methylat
595 Systems Biology and Medicine 201
596 GZeng Y, Chen T. DNA methylation
597 2019;10:257.
598 7 SanMiguel JM, Bartolomei MS. DN
699 development. B
-
- Systems Biology and Medicine 2010;2:362–71.
595 Systems Biology and Medicine 2010;2:362–71.
596 Eeng Y, Chen T. DNA methylation reprogramming during mammalian development. *Genes*
597 2019;10:257.
598 7SanMiguel JM, Bartol 595 Systems Biology and Medicine 2010,2:302 71.
596 6Zeng Y, Chen T. DNA methylation reprogrammi
597 7SanMiguel JM, Bartolomei MS. DNA methylatio
599 development. *Biol Reprod* 2018;99:252–62. htt
600 8Tucci V, Isles AR, K 597 6 2019;10:257.
596 7 San Miguel JM, Bartolomei MS. DNA methylation dynamics of genomic imprinting in mouse
599 development. *Biol Reprod* 2018;99:252–62. https://doi.org/10.1093/biolre/ioy036.
600 8 Tucci V, Isles AR,
-
- 598 7SanMiguel JM
599 development.
600 8Tucci V, Isles 4
601 imprinting and
602 9Piedrahita JA.
603 *Clin Mol Terat* 598 7 San Miguel JM, Bartolomei MS. DNA methylation dynamics of genomic imprinting in mouse
599 development. *Biol Reprod* 2018;99:252–62. https://doi.org/10.1093/biolre/ioy036.
600 8 Tucci V, Isles AR, Kelsey G, Ferguson-
- Separator and the production of the production of the production of the production of the processes in mammals. Cell 2019;176:952–65.

9 Piedrahita JA. The role of imprinted genes in fetal growth abnormalities. *Birth Defe* 600 8 Tucci V, Isles Art, Kelsey G, Ferguson-Smith Ac, Bartolomer MS, Benvenisty N, et al. Genomic
601 imprinting and physiological processes in mammals. *Cell* 2019;176:952–65.
603 *Clin Mol Teratol* 2011;91:682–92. https 602 9 Piedrahita JA. The role of imprinted genes in fietal growth abnormalities. *Bir*
603 *Clin Mol Teratol* 2011;91:682–92. https://doi.org/10.1002/bdra.20795.
604 10 Moore GE, Ishida M, Demetriou C, Al-Olabi L, Leon LJ, Clin Mol Teratol 2011;91:682–92. https://doi.org/10.1002/bdra.20795.

603 Clin Mol Teratol 2011;91:682–92. https://doi.org/10.1002/bdra.20795.

604 10 Moore GE, Ishida M, Demetriou C, Al-Olabi L, Leon LJ, Thomas AC, *et al* 603 Clin Mor Ferator 2011;91:682–92. https://doi.org/10.1002/bdra.20795.
604 10 Moore GE, Ishida M, Demetriou C, Al-Olabi L, Leon LJ, Thomas AC,
605 interaction of imprinted genes in human fetal growth. *Philos Trans R So* 10 Moore GE, Island M, Demetriou C, Al-Olabi L, Leon D, Thomas AC, et al. The role and

10 Interaction of imprinted genes in human fetal growth. *Philos Trans R Soc Lond B Biol Sci*

10 2015;**370**:20140074. https://doi.org
-
-
- meraction of imprinted genes in human fetal growth: *Thilos Trans R Soc Lond B Biol Sci*

606 2015;**370**:20140074. https://doi.org/10.1098/rstb.2014.0074.

607 11 Jima DD, Skaar DA, Planchart A, Motsinger-Reif A, Cevik SE, 11 Jima DD, Skaar DA, Planchart A, Motsinger-Reif A, Cevik S
608 of candidate human imprint control regions: the imprintome. *E*
609 12 Horsthemke B. Mechanisms of Imprint Dysregulation.
610 13 Faulk C, Dolinoy DC. Timing
- 608 of candidate human imprint control regions: the imprintome. *Epigenetics* 2022:1–24.
609 12 Horsthemke B. Mechanisms of Imprint Dysregulation.
610 13 Faulk C, Dolinoy DC. Timing is everything: the when and how of envir 609 12 Horsthemke B. Mechanisms of Imprint Dysregulation.
609 12 Horsthemke B. Mechanisms of Imprint Dysregulation.
610 13 Faulk C, Dolinoy DC. Timing is everything: the when and how of environmentally
611 changes in the e 610 13 Faulk C, Dolinoy DC. Timing is everything: the when an
611 changes in the epigenome of animals. *Epigenetics* 2011;6:79
612 14 Angers B, Castonguay E, Massicotte R. Environmentally
613 methylation: how to deal with 611 changes in the epigenome of animals. *Epigenetics* 2011;6:791–7.
612 14 Angers B, Castonguay E, Massicotte R. Environmentally induced phenotypes and DNA
613 methylation: how to deal with unpredictable conditions until 612 changes in the epigenome of animals. Epigenetics 2011, 6:791–7.
612 14 Angers B, Castonguay E, Massicotte R. Environmentally indu
613 methylation: how to deal with unpredictable conditions until the
616 Molecular Ecolo 613 methylation: how to deal with unpredictable conditions until the next generation and after.
614 Molecular Ecology 2010;19:1283–95.
615 15 Senut M-C, Cingolani P, Sen A, Kruger A, Shaik A, Hirsch H, et al. Epigenetics o
- 614 *Molecular Ecology* 2010;19:1283–95.
615 15 Senut M-C, Cingolani P, Sen A, Kruger A, Shaik A, Hirsch H, *et al.* Epigenetics of early-lif
616 lead exposure and effects on brain development. *Epigenomics* 2012;4:665–74. 615 15 Senut M-C, Cingolani P, Sen A, K
616 lead exposure and effects on brain de
617 https://doi.org/10.2217/epi.12.58.
618 16 Parsanathan R, Karundevi B. Phi
619 impairs insulin signalling. Journal of El
620 https://doi.
-
- 615 15 Senut M-C, Cingolani P, Sen A, Kruger A, Shaik A, Hirsch H, et al. Epigenetics of early-life
-
- 617 https://doi.org/10.2217/epi.12.58.
618 16 Parsanathan R, Karundevi B. Phthalate exposure in utero causes epigenet
619 impairs insulin signalling. Journal of Endocrinology 2014;223:47–66.
620 https://doi.org/10.1530/JOE 618 16 Parsanathan R, Karundevi B. F
619 impairs insulin signalling. Journal of
620 https://doi.org/10.1530/JOE-14-01
621 17 Dignam T, Kaufmann RB, LeSt
622 United States, 1970-2017: Public He
623 Exposure. J Public Health impairs insulin signalling. Journal of Endocrinology 2014;223:47–66.

620 https://doi.org/10.1530/JOE-14-0111.

621 17 Dignam T, Kaufmann RB, LeStourgeon L, Brown MJ. Control of Lead Sources in the

622 United States, 1970 620 https://doi.org/10.1530/JOE-14-0111.
621 17 Dignam T, Kaufmann RB, LeStourgeon L, Brown MJ. Control of
622 United States, 1970-2017: Public Health Progress and Current Challe
623 Exposure. *J Public Health Manag Pract* 621 17 Dignam T, Kaufmann RB, LeStour
622 United States, 1970-2017: Public Healt
623 Exposure. *J Public Health Manag Pract*
624 https://doi.org/10.1097/PHH.0000000
625 18 Zhou F, Yin G, Gao Y, Liu D, Xie J,
626 and lactat United States, 1970-2017: Public Health Progress and Current Challenges to Eliminating I

Exposure. J Public Health Manag Pract 2019;25:S13–22.

https://doi.org/10.1097/PHH.000000000000889.

525 18 Zhou F, Yin G, Gao Y, Li Exposure. J Public Health Manag Pract 2019; **25**: S13–22.

624 https://doi.org/10.1097/PHH.0000000000000889.

625 18 Zhou F, Yin G, Gao Y, Liu D, Xie J, Ouyang L, *et al.* Toxicity assessment due to prenatal

and lactatio
-
- 623 Exposure. J. Fublic Health Manag Pract 2019,23.313–22.
624 https://doi.org/10.1097/PHH.0000000000000889.
625 18 Zhou F, Yin G, Gao Y, Liu D, Xie J, Ouyang L, et al. To
626 and lactational exposure to lead, cadmium and 625 18 Zhou F, Yin G, Gao Y, Liu D, Xie J, Ouyang L, et
626 and lactational exposure to lead, cadmium and mer
627 2019;133:105192. https://doi.org/10.1016/j.envint
- 625 18 Zhou F, Fin G, Gao F, Eta D, Xie J, Ouyang L, et al. Toxicity assessment due to prenatal
626 and lactational exposure to lead, cadmium and mercury mixtures. *Environ Int*
627 2019;133:105192. https://doi.org/10.101
- 627 and lactational exposure to lead, cadmium and mercury mixtures. *Environ Int*ernational exposure to lead, cadmium and mercury mixtures. *Environ International* 2019;133:105192. https://doi.org/10.1016/j.envint.2019.10 $\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{L}(\mathcal{L})=\mathcal{$

19 High-precision Pb isotopes of drinking water lead pipes: Implications for human exposure

industrial Pb in the United States. *Sci Total Environ* 2023; **871**:162067.

https://doi.org/10.1016/j.scitotenv.2023.162067.

20 Di industrial Pb in the United States. Sci Total Environ 2023; 871:162067.

https://doi.org/10.1016/j.scitotenv.2023.162067.

20 Dietrich M, Barlow CF, Entwistle JA, Meza-Figueroa D, Dong C, Gunkel-Grillon P, et al.

21 Predi 631 https://doi.org/10.1016/j.scitotenv.2023.162067.
632 20 Dietrich M, Barlow CF, Entwistle JA, Meza-Figueroa D, Dong C, G
633 Predictive modeling of indoor dust lead concentrations: Sources, risks
634 intervention. *Envi* 632 20 Dietrich M, Barlow CF, Entwistle JA, Meza-Fi
633 Predictive modeling of indoor dust lead concentra
634 intervention. *Environ Pollut* 2023;**319**:121039. http
635 21 Wang Y, Qian H. Phthalates and Their Impac
636 20 Example 1. Entertainment of the term of the intervention. *Environ Pollut* 2023;319:121039. https://doi.org/10.1016/j.envpol.2023.12103

1635 21 Wang Y, Qian H. Phthalates an 634 intervention. *Environ Pollut* 2023;319:121039. https://doi.org/10.1016/j.envpol.2023.1
635 21 Wang Y, Qian H. Phthalates and Their Impacts on Human Health. *Healthcare (Bas*
636 2021;9:603. https://doi.org/10.3390/he mervention. Environ Pollut 2023;319:121039. https://doi.org/10.1016/j.envpol.2023.121035.

635 21 Wang Y, Qian H. Phthalates and Their Impacts on Human Health. Healthcare (Basel)

636 2021;9:603. https://doi.org/10.3390/he 21 Wang T, Qian H. Findrates and Their Impacts on Human Health. Healthcare (Basel)

2021;9:603. https://doi.org/10.3390/healthcare9050603.

637 22 Lin Y, Wei J, Li Y, Chen J, Zhou Z, Song L, *et al.* Developmental exposure 22 Lin Y, Wei J, Li Y, Chen J, Zhou Z, Song L, et al. Develof
638 ethylhexyl) phthalate impairs endocrine pancreas and lead
639 glucose homeostasis in the rat. American Journal of Physic
640 2011;301:E527-38. https://doi.o 638 ethylhexyl) phthalate impairs endocrine pancreas and leads to long-term adverse eff
638 glucose homeostasis in the rat. American Journal of Physiology-Endocrinology and M
640 2011;301:E527-38. https://doi.org/10.1152/ ethylhexyl) phthalate in the rat. American Journal of Physiology-Endocrinology and Metabolis

640 2011;301:E527-38. https://doi.org/10.1152/ajpendo.00233.2011.

641 23 Erythropel HC, Maric M, Nicell JA, Leask RL, Yargeau V glucose homeostasis in the rat. American Journal of Physiology-Endocrinology and Metabolism

640 2011;301:E527-38. https://doi.org/10.1152/ajpendo.00233.2011.

641 23 Erythropel HC, Maric M, Nicell JA, Leask RL, Yargeau V. Experimental Contracts of Maria Microphology and the present of A1 23 Erythropel HC, Maric M, Nicell JA, Leask RL, Yargeau V. Leach
642 ethylhexyl)phthalate (DEHP) from plastic containers and the questi
643 Appl Microbiol ethylhexyl)phthalate (DEHP) from plastic containers and the question of human exposure.

Appl Microbiol Biotechnol 2014;98:9967–81. https://doi.org/10.1007/s00253-014-6183-8.

24 Wang T, Pehrsson EC, Purushotham D, Li D, Z 643 Appl Microbiol Biotechnol 2014;98:9967–81. https://doi.org/10.1007/s00253-014-6183-8.
644 24 Wang T, Pehrsson EC, Purushotham D, Li D, Zhuo X, Zhang B, et al. The NIEHS TaRGET
645 Consortium and environmental epigenomi 644 24 Mang T, Pehrsson EC, Purushotham D, Li D, Zhuo X, Zhang B, et al. The NIEHS TaRGET
645 Consortium and environmental epigenomics. Nature Biotechnology 2018;36:225–7.
646 25 Dou JF, Farooqui Z, Faulk CD, Barks AK, Jon 645 Consortium and environmental epigenomics. Nature Biotechnology 2018;36:225–7.
646 25 Dou JF, Farooqui Z, Faulk CD, Barks AK, Jones T, Dolinoy DC, *et al.* Perinatal Lead (Pb)
647 Exposure and Cortical Neuron-Specific D 646 25 Dou JF, Farooqui Z, Faulk CD, Barks AK, Jones T, Dolinoy DC, et al. Perinatal Lea
647 Exposure and Cortical Neuron-Specific DNA Methylation in Male Mice. *Genes (Basel)*
648 2019;10:E274. https://doi.org/10.3390/gen Exposure and Cortical Neuron-Specific DNA Methylation in Male Mice. *Genes (Basel)*

2019;10:E274. https://doi.org/10.3390/genes10040274.

26 Faulk C, Barks A, Liu K, Goodrich JM, Dolinoy DC. Early-life lead exposure resul Exposure and Cortical Neuron-Specific DNA Methylation in Male Mice. Senes (Duser)

2019;10:E274. https://doi.org/10.3390/genes10040274.

369 and sex-specific effects on weight and epigenetic gene regulation in weanling mic 26 Faulk C, Barks A, Liu K, Goodrich JM, Dolinoy DC. Ea

and sex-specific effects on weight and epigenetic gene re

651 Epigenomics 2013;5:487–500. https://doi.org/10.2217/e

652 27 Schmidt J-S, Schaedlich K, Fiandanese N, Epigenomics 2013;5:487–500. https://doi.org/10.2217/epi.13.49.

652 27 Schmidt J-S, Schaedlich K, Fiandanese N, Pocar P, Fischer B. Effects of di(2-eth)

653 phthalate (DEHP) on female fertility and adipogenesis in C3H/N m Epigenomics 2013,3:487–500. https://doi.org/10.2217/epi.13.43.
652 27 Schmidt J-S, Schaedlich K, Fiandanese N, Pocar P, Fischer B. I
653 phthalate (DEHP) on female fertility and adipogenesis in C3H/N m
654 2012;120:1123-9. 653 phthalate (DEHP) on female fertility and adipogenesis in C3H/N mice. *Environ Health Perspe*
654 2012;120:1123-9. https://doi.org/10.1289/ehp.1104016.
655 28 Neier K, Cheatham D, Bedrosian LD, Dolinoy DC. Perinatal exp 653 phthalate (DEHT) on female fertility and adipogenesis in C3H/N mice. Environ freditify respect
654 2012;120:1123-9. https://doi.org/10.1289/ehp.1104016.
655 28 Neier K, Cheatham D, Bedrosian LD, Dolinoy DC. Perinatal e 264 2019;120:1123–9. https://doi.org/10.1017/S204017441800
656 phthalate mixtures result in sex-specific effects on body w
657 intracisternal A-particle (IAP) DNA methylation in weanling
658 2019;10:176–87. https://doi.org 655 28 Neier K, Cheatham D, Bedrosian LD, Dolinoy DC. Perinatal exposures to phthalates and
656 phthalate mixtures result in sex-specific effects on body weight, organ weights and of the mixtures result in sex-specific effects on body weight, organ weights and

intracisternal A-particle (IAP) DNA methylation in weanling mice. *J Dev Orig Health Dis*

2019;10:176–87. https://doi.org/10.1017/S20401744 intracisternal A-particle (IAP) DNA methylation in weanling mice. J Dev Orig Health 1

658 2019;10:176–87. https://doi.org/10.1017/S2040174418000430.

659 29 Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M 658 2019;10:176-87. https://doi.org/10.1017/S2040174418000430.
658 2019;10:176-87. https://doi.org/10.1017/S2040174418000430.
660 guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol* 2020;18:e3
661 2019 Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Ba

guidelines 2.0: Updated guidelines for reporting animal research.

https://doi.org/10.1371/journal.pbio.3000410.

30 Svoboda LK, Neier K, Wang K, Cavalcante guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol* 2020;18:e3000
661 https://doi.org/10.1371/journal.pbio.3000410.
662 30 Svoboda LK, Neier K, Wang K, Cavalcante RG, Rygiel CA, Tsai Z, *et al.* guidelines 2.0: Opdated guidelines for reporting animal research. PLOS Biol 2020,18:e3000410.

https://doi.org/10.1371/journal.pbio.3000410.

Societic programming of dna methylation by perinatal lead exposure: implications 662 30 Svoboda LK, Neier K, Wang K, Cavalcante
663 specific programming of dna methylation by per
664 environmental epigenetics studies. *Epigenetics*
665 https://doi.org/10.1080/15592294.2020.18418
666 31 Andrews. *FastQC* Society Sociological LK, Weier K, Wang K, Cavalcante KO, Nygiel CA, Tsai 2, et al. Tissue and sex-

specific programming of dna methylation by perinatal lead exposure: implications for

environmental epigenetics studies. 664 environmental epigenetics studies. Epigenetics 2021;16:1102-22.
665 https://doi.org/10.1080/15592294.2020.1841872.
666 31 Andrews. FastQC A Quality Control tool for High Throughput Sequence Data. 201
667 https://www.bi environmental epigenetics studies. *Epigenetics 2021*, 10.1102–22.

665 https://doi.org/10.1080/15592294.2020.1841872.

666 31 Andrews. *FastQC A Quality Control tool for High Throughput*

https://www.bioinformatics.babrah mathers, and the set of a https://www.bioinformatics.babraham.ac.uk/proje
668 32 Ewels P, Magnusson M, Lundin S, Käller M. M
669 multiple tools and samples in a single report. *Bioinf*
6 667 https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (Accessed 15 February 2023).
668 32 Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: summarize analysis results for
669 multiple tools and samples in a singl 668 32 Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* 2016;32:3047–8.
670 https://doi.org/10.1093/bioinformatics/btw354. 669 multiple tools and samples in a single report. *Bioinformatics* 2016;**32**:3047–8.
670 https://doi.org/10.1093/bioinformatics/btw354. 669 matter tools and samples in a single report. Biomformatics 2016;32:3047–8.
670 https://doi.org/10.1093/bioinformatics/btw354. \mathcal{G}_0 is the state of the state \mathcal{G}_0 is the state of the state \mathcal{G}_0

672 Maleger F. Him Galore. 2015. OKL.
673 2023).
674 34 Krueger F, Andrews SR. Bismark: a 1
675 Seq applications. *Bioinformatics* 2011;27:
676 https://doi.org/10.1093/bioinformatics/t
677 35 Langmead B, Salzberg SL. Fast

-
- 673 2023).
674 34 Krueger F, Andrews SR. Bismark: a flexible aligner and methylation caller for Bisulfite-
675 Seq applications. *Bioinformatics* 2011;27:1571–2.
676 https://doi.org/10.1093/bioinformatics/btr167.
677 35 L 674 34 K
 675 Seq ap
 676 https:/
 677 35 L
 678 2012;9
 679 36 J
- 680 sensitive calling of differentially methylated regions from bisulfite sequencing data. Genome 681 Res 2016;26:256–62. https://doi.org/10.1101/gr.196394.115. 676 Seq applications. Biomformatics 2011,27:1571–2.
676 https://doi.org/10.1093/bioinformatics/btr167.
677 35 Langmead B, Salzberg SL. Fast gapped-read a
678 2012;9:357–9. https://doi.org/10.1038/nmeth.19.
679 36 Jühling 677 35 Langmead B, Salzberg SL. Fast gapped-read
678 2012;9:357-9. https://doi.org/10.1038/nmeth.1
679 36 Jühling F, Kretzmer H, Bernhart SH, Otto C,
680 sensitive calling of differentially methylated regional Res 2016;26: 678 2012;9:357–9. https://doi.org/10.1038/nmeth.1923.
679 36 Jühling F, Kretzmer H, Bernhart SH, Otto C, Stadler PF, Hoffmann S. metilene: fast a
680 sensitive calling of differentially methylated regions from bisulfite se 679 36 Jühling F, Kretzmer H, Bernhart SH, Otto C, Stad
680 sensitive calling of differentially methylated regions fi
681 *Res* 2016;26:256–62. https://doi.org/10.1101/gr.196:
682 37 Park Y, Figueroa ME, Rozek LS, Sartor M 680 sensitive calling of differentially methylated regions from bisulfite sequencing data. *Genome*
681 *Res* 2016;26:256–62. https://doi.org/10.1101/gr.196394.115.
682 37 Park Y, Figueroa ME, Rozek LS, Sartor MA. MethylSi
- 681 Res 2016;26:256–62. https://doi.org/10.1101/gr.196394.115.
682 Brack Y, Figueroa ME, Rozek LS, Sartor MA. MethylSig: a whole genome DNA methylatio
683 analysis pipeline. *Bioinformatics* 2014;30:2414–22.
684 https://do 682 37 Park Y, Figueroa ME, Rozek LS, Sartor MA. MethylSig: a v
683 analysis pipeline. *Bioinformatics* 2014;30:2414–22.
684 https://doi.org/10.1093/bioinformatics/btu339.
685 38 Cavalcante RG, Sartor MA. annotatr: genomic
-

- analysis pipeline. *Bioinformatics* 2014;30:2414–22.

https://doi.org/10.1093/bioinformatics/btu339.

685 38 Cavalcante RG, Sartor MA. annotatr: genomic regions in context. *Bioinformatics*

686 2017;33:2381–3. https://doi 684 https://doi.org/10.1093/bioinformatics/btu339.
685 38 Cavalcante RG, Sartor MA. annotatr: genomic
686 2017;33:2381–3. https://doi.org/10.1093/bioinforn
687 39 Welch RP, Lee C, Imbriano PM, Patil S, Weyme
688 set enrich 685 38 Cavalcante RG, Sartor MA. annotatr: genor
686 2017;33:2381–3. https://doi.org/10.1093/bioinformatics/beam welch RP, Lee C, Imbriano PM, Patil S, Wey
688 set enrichment testing for ChIP-seq data. Nucleic
689 https:// 686 2017;33:2381–3. https://doi.org/10.1093/bioinformatics/btx183.
686 2017;33:2381–3. https://doi.org/10.1093/bioinformatics/btx183.
687 39 Welch RP, Lee C, Imbriano PM, Patil S, Weymouth TE, Smith RA, *et al.* ChIP-Enric 2017;33:2381–3. https://doi.org/10.1093/bioinformatics/btx183.

687 39 Welch RP, Lee C, Imbriano PM, Patil S, Weymouth TE, Smith RA, *et al.* ChIP-Enrich: gene

set enrichment testing for ChIP-seq data. *Nucleic Acids Res*
-
-
- 688 set enrichment testing for ChIP-seq data. *Nucleic Acids Res* 2014;42:e105.
689 https://doi.org/10.1093/nar/gku463.
690 40 Williamson CM, Blake A, Thomas S, Beechey CV, Hancock J, Cattanach BM, *et al.* World
691 Wide 689 set enrichment testing for ChIP-seq data. Nucleic Acids Res 2014;42:e105.
689 https://doi.org/10.1093/nar/gku463.
690 40 Williamson CM, Blake A, Thomas S, Beechey CV, Hancock J, Cattanac
691 Wide Web Site-Mouse Imprint 690 40 Williamson CM, Blake A, Thoma
691 Wide Web Site-Mouse Imprinting Dat
692 41 Tucci V, Isles AR, Kelsey G, Fergu
693 Imprinting and Physiological Processe
694 https://doi.org/10.1016/j.cell.2019.01
695 42 Juan AM, Foo 691 Williamson CM, Blake A, Thomas S, Beechey Cv, Trancock 3, Cattanach BM, et al. World
691 Wide Web Site-Mouse Imprinting Data and References. *Oxfordshire: MRC Hartwell* 2013.
693 Imprinting and Physiological Processes 692 data and References. Oxfordshire: MRC Hartwell 2013.
692 data in References. Oxfordshire: MRC Hartolomei MS, et al. Genon
693 imprinting and Physiological Processes in Mammals. Cell 2019;176:952–65.
694 https://doi.org
- 692 41 Tucci V, Isles AR, Reisey G, Ferguson-Smith Ac, Tucci V, Bartolomer MS, et al. Genomic
693 Imprinting and Physiological Processes in Mammals. *Cell* 2019;176:952–65.
694 https://doi.org/10.1016/j.cell.2019.01.043.
6 694 https://doi.org/10.1016/j.cell.2019.01.043.
695 42 Juan AM, Foong YH, Thorvaldsen JL, Lan Y, Leu NA, Rurik JG, *et al*. Tissue-specific
696 Grb10/Ddc insulator drives allelic architecture for cardiac development. *Mol*
-
- 699 parental DNA methylomes in mammals. *Cell* 2014;157:979–91.
100 https://doi.org/10.1016/j.cell.2014.04.017.
101 44 Riemondy KA, Sheridan RM, Gillen A, Yu Y, Bennett CG, Hesselberth JR. valr: 696 Grb10/Ddc insulator drives allelic architecture for cardiac development. *Mol Cell*
696 Grb10/Ddc insulator drives allelic architecture for cardiac development. *Mol Cell*
697 2022;82:3613-3631.e7. https://doi.org/10.1 697 2022;82:3613-3631.e7. https://doi.org/10.1016/j.molcel.2022.08.021.
698 43 Wang L, Zhang J, Duan J, Gao X, Zhu W, Lu X, *et al.* Programming and inheri
699 parental DNA methylomes in mammals. *Cell* 2014;157:979-91.
70 43 Wang L, Zhang J, Duan J, Gao X, Zhu W, Lu X, et al. Programming
699 parental DNA methylomes in mammals. *Cell* 2014;157:979-91.
700 https://doi.org/10.1016/j.cell.2014.04.017.
701 44 Riemondy KA, Sheridan RM, Gillen A, 699 parental DNA methylomes in mammals. Cell 2014;157:979-91.
699 parental DNA methylomes in mammals. Cell 2014;157:979-91.
701 44 Riemondy KA, Sheridan RM, Gillen A, Yu Y, Bennett CG, Hesselberth JR. valr:
702 Reproducib
-
-
-
-
- 699 parental DNA methylomes in mammals. Cell 2014,157:979–91.

700 https://doi.org/10.1016/j.cell.2014.04.017.

701 44 Riemondy KA, Sheridan RM, Gillen A, Yu Y, Bennett CG, He

702 Reproducible genome interval analysis in 701 44 Riemondy KA, Sheridan RM, Gillen A,
702 Reproducible genome interval analysis in R.
703 https://doi.org/10.12688/f1000research.11
704 45 Dolinoy DC, Weidman JR, Jirtle RL. Epi
705 developmental environment to adult Reproducible genome interval analysis in R. F1000Res 2017;6:1025.

702 Reproducible genome interval analysis in R. F1000Res 2017;6:1025.

703 https://doi.org/10.12688/f1000research.11997.1.

705 developmental environment t To the metropology of the metropology of the same interval analysis in R. F1000Res 2017;6:1025.

703 https://doi.org/10.12688/f1000research.11997.1.

704 45 Dolinoy DC, Weidman JR, Jirtle RL. Epigenetic gene regulation:

7 704 45 Dolinoy DC, Weidman JR, Jirtle RL. Epigenetic
705 developmental environment to adult disease. *Repr*
706 https://doi.org/10.1016/j.reprotox.2006.08.012.
707 46 Thomason ME, Hect JL, Rauh VA, Trentacosta
708 Prenatal 2015 developmental environment to adult disease. *Reprod Toxicol* 2007;23:297-307.

206 https://doi.org/10.1016/j.reprotox.2006.08.012.

207 46 Thomason ME, Hect JL, Rauh VA, Trentacosta C, Wheelock MD, Eggebrecht

208 Pre
-
- 205 developmental environment to adult disease. Reprod Toxicol 2007;23:297–307.

706 https://doi.org/10.1016/j.reprotox.2006.08.012.

707 46 Thomason ME, Hect JL, Rauh VA, Trentacosta C, Wheelock MD, Eggebrech

708 Prenata 707 46 Thomason ME, Hect JL, Rauh VA, Trentacos
708 Prenatal lead exposure impacts cross-hemispheri
709 fetal brain. *Neuroimage* 2019;191:186–92.
710 https://doi.org/10.1016/j.neuroimage.2019.02.0
711 47 Neier K, Montrose Franch Thomason ME, Hect J.C, Nauli VA, Trentacosta C, Wheelock MD, Eggebrecht AT, et al.

708 Prenatal lead exposure impacts cross-hemispheric and long-range connectivity in the huma

709 fetal brain. *Neuroimage* 2019;19 709 fetal brain. *Neuroimage* 2019;191:186–92.
710 https://doi.org/10.1016/j.neuroimage.2019.02.017.
711 47 Neier K, Montrose L, Chen K, Malloy MA, Jones TR, Svoboda LK, *et al.* Short- and long-
712 term effects of perina
- 710 https://doi.org/10.1016/j.neuroimage.2019
711 47 Neier K, Montrose L, Chen K, Malloy I
712 term effects of perinatal phthalate exposur
713 *Environ Epigenet* 2020;6:dvaa017. https://d
- 711 47 Neier K, Montrose L, Chen K, Malloy MA, Jones
712 term effects of perinatal phthalate exposures on me
713 *Environ Epigenet* 2020;6:dvaa017. https://doi.org/10
- 711 47 Neier K, Montrose L, Chen K, Malloy MA, Jones TR, Svoboda LK, et al. Short- and long-
712 term effects of perinatal phthalate exposures on metabolic pathways in the mouse liver.
713 Environ Epigenet 2020;6:dvaa017.
- 713 Environ Epigenet 2020;6:dvaa017. https://doi.org/10.1093/eep/dvaa017. 713 Environ Epigenet 2020;6:dvaa017. https://doi.org/10.1093/eep/dvaa017.

48 et al. Prenatal Lead (Pb) Exposure and Peripheral Blood DNA Methylation (5mC) and

716 Hydroxymethylation (5hmC) in Mexican Adolescents from the ELEMENT Birth Cohort. *Enviror*

717 Health Perspect 2021;129:67002. https:// Profile and Periodial Lead (Pb) Exposure and Peripheral Blood DNA Methylation (5mc) and

716 Hydroxymethylation (5hmC) in Mexican Adolescents from the ELEMENT Birth Cohort

717 Health Perspect 2021;129:67002. https://doi.o 717 Health Perspect 2021;129:67002. https://doi.org/10.1289/EHP8507.
718 49 Chen C-H, Jiang SS, Chang I-S, Wen H-J, Sun C-W, Wang S-L. Association between fetal
719 exposure to phthalate endocrine disruptor and genome-wide 717 Thealth Perspect 2021,129:67002. https://doi.org/10.1269/EHP8507.
718 49 Chen C-H, Jiang SS, Chang I-S, Wen H-J, Sun C-W, Wang S-L. Ass
719 exposure to phthalate endocrine disruptor and genome-wide DNA m
720 Environ Re exposure to phthalate endocrine disruptor and genome-wide DNA methylation at birth.

T20 Environ Res 2018;162:261–70. https://doi.org/10.1016/j.envres.2018.01.009.

T21 50 Sobolewski M, Varma G, Adams B, Anderson DW, Schne Find The Past 2018;162:261-70. https://doi.org/10.1016/j.envres.2018.01.009.

T21 50 Sobolewski M, Varma G, Adams B, Anderson DW, Schneider JS, Cory-Slechta DA.

Developmental Lead Exposure and Prenatal Stress Result in Se 720 Environ Res 2018;162:261–70. https://doi.org/10.1016/j.envres.2018.01.01.009.
721 50 Sobolewski M, Varma G, Adams B, Anderson DW, Schneider JS, Cory-Sle
722 Developmental Lead Exposure and Prenatal Stress Result in Sex The Developmental Lead Exposure and Prenatal Stress Result in Sex-Specific Reprograming

723 Adult Stress Physiology and Epigenetic Profiles in Brain. Toxicol Sci 2018;163:478–89.

724 https://doi.org/10.1093/toxsci/kfy046 723 Adult Stress Physiology and Epigenetic Profiles in Brain. Toxicol Sci 2018;163:478–89.
724 https://doi.org/10.1093/toxsci/kfy046.
725 51 Liu S, Wang K, Svoboda LK, Rygiel CA, Neier K, Jones TR, *et al.* Perinatal DEHP 723 Adult Stress Physiology and Epigenetic Profiles in Brain. Toxicol Scr 2018;163:478–89.
724 https://doi.org/10.1093/toxsci/kfy046.
725 51 Liu S, Wang K, Svoboda LK, Rygiel CA, Neier K, Jones TR, *et al.* Perinatal DEHP 725 51 Liu S, Wang K, Svoboda LK, Rygiel
726 induces sex- and tissue-specific DNA me
727 Environ Epigenet 2021;7:dvab004. https:
728 52 Svoboda LK, Ishikawa T, Dolinoy D
729 effects on epigenetic programming and
730 Epigen France States of the S, Wang K, Svoboda LK, Rygiel CA, Neier K, Sones TR, et al. Permatal DEHP exposure

induces sex- and tissue-specific DNA methylation changes in both juvenile and adult mice.

For Environ Epigenet 2021; Fortion Epigenet 2021;7:dvab004. https://doi.org/10.1093/eep/dvab004.

T28 52 Svoboda LK, Ishikawa T, Dolinoy DC. Developmental toxicant exposures and sex-spec

effects on epigenetic programming and cardiovascular health a 727 Environ Epigenet 2021,7:dvaboo4: https://doi.org/10.1093/eep/dvaboo4:
728 S2 Svoboda LK, Ishikawa T, Dolinoy DC. Developmental toxicant exposureffects on epigenetic programming and cardiovascular health across gener
73 229 effects on epigenetic programming and cardiovascular health across generations. *Environ*

230 *Epigenet* 2022;8:dvac017. https://doi.org/10.1093/eep/dvac017.

231 Singh G, Singh V, Sobolewski M, Cory-Slechta DA, Schne Frances on epigenetic programming and cardiovascular health across generations. Environt

730 Epigenet 2022;8:dvac017. https://doi.org/10.1093/eep/dvac017.

731 S3 Singh G, Singh V, Sobolewski M, Cory-Slechta DA, Schneider 730 Epigenet 2022, 3:dvaco17. https://doi.org/10.1093/eep/dvaco17.
731 53 Singh G, Singh V, Sobolewski M, Cory-Slechta DA, Schneider
732 Developmental Lead Exposure on the Brain. *Front Genet* 2018;9:8
733 https://doi.org/ Developmental Lead Exposure on the Brain. Front Genet 2018;9:89.

733 https://doi.org/10.3389/fgene.2018.00089.

734 54 Smallwood SA, Tomizawa S-I, Krueger F, Ruf N, Carli N, Segonds-Pichon A, et al. Dynamic

735 CpG islan Exposure on the Brain. From Genet 2018,9:89.

733 https://doi.org/10.3389/fgene.2018.00089.

734 54 Smallwood SA, Tomizawa S-I, Krueger F, Ruf N, Carli N, Segond

735 CpG island methylation landscape in oocytes and preimpl 734 54 Smallwood SA, Tomizawa S-I, Krueger
735 CpG island methylation landscape in oocytes
736 2011;43:811–4. https://doi.org/10.1038/ng.
737 55 Kang E-R, Iqbal K, Tran DA, Rivas GE, Si
738 disruptors on imprinted gene ex 734 54 Smallwood SA, Tomizawa S-I, Krueger F, KuTV, Carli N, Segonds-Fichon A, et al. Dynamic

735 CpG island methylation landscape in oocytes and preimplantation embryos. Nat Genet

736 2011;43:811–4. https://doi.org/10.1 2011;43:811–4. https://doi.org/10.1038/ng.864.

2011;43:811–4. https://doi.org/10.1038/ng.864.

737 S5 Kang E-R, Iqbal K, Tran DA, Rivas GE, Singh P, Pfeifer GP, et al. Effects of endocrine

disruptors on imprinted gene ex 2012 2011;31:206–13.

737 55 Kang E-R, Iqbal K, Tran DA, Rivas GE, Singh I

disruptors on imprinted gene expression in the m

739 https://doi.org/10.4161/epi.6.7.16067.

740 56 Krishnamoorthy M, Gerwe BA, Scharer CD,

741 737 55 Kang E-R, Iqbal K, Tran DA, Itwas GE, Singh T, Trenet GT, et al. Effects of endocrine

738 disruptors on imprinted gene expression in the mouse embryo. *Epigenetics* 2011;6:937-

740 56 Krishnamoorthy M, Gerwe BA, S 238 disruptors on imprinted gene expression in the mouse embryo. Epigenetics 2011,0:537–50.

239 https://doi.org/10.4161/epi.6.7.16067.

240 56 Krishnamoorthy M, Gerwe BA, Scharer CD, Heimburg-Molinaro J, Gregory F, Nash R 740 56 Krishnamoorthy M, Gerwe BA, Sch
741 *al.* GABRB3 gene expression increases u
742 *J Recept Signal Transduct Res* 2011;31:2
743 https://doi.org/10.3109/10799893.2011
744 57 Bastepe M, Fröhlich LF, Hendy GN
745 Autoso 740 56 Krishnamoorthy M, Gerwe BA, Scharer CD, Heimburg-Molinaro 3, Gregory F, Nash RJ, et

741 al. GABRB3 gene expression increases upon ethanol exposure in human embryonic stem cells.

742 *J Recept Signal Transduct Res* 742 *I. GABRB3 gene expression increases upon ethanol exposure in human embryonic stem cells.*

742 *J. Recept Signal Transduct Res* 2011;31:206–13.

743 https://doi.org/10.3109/10799893.2011.569723.

745 Autosomal dominan 742 J Recept Signal Transduct Res 2011, 31:200 13.

743 https://doi.org/10.3109/10799893.2011.56972

744 57 Bastepe M, Fröhlich LF, Hendy GN, Indrid:

745 Autosomal dominant pseudohypoparathyroidis

747 2003;112:1255–63. h 744 57 Bastepe M, Fröhlich LF, Hendy GN, Indridasc
745 Autosomal dominant pseudohypoparathyroidism
746 microdeletion that likely disrupts a putative imprir
747 2003;112:1255–63. https://doi.org/10.1172/JCl19
748 58 Eggerma 744 57 Bastepe M, Fröhlich LF, Hendy GN, Indridason OS, Josse Rd, Rosinyama H, et al.
745 Autosomal dominant pseudohypoparathyroidism type Ib is associated with a heterozy
746 microdeletion that likely disrupts a putative Autosomal dominant pseudohypoparathyroidism type Ib is associated with a heterozygous

T46 microdeletion that likely disrupts a putative imprinting control element of *GNAS. J Clin Invest*

2003;112:1255–63. https://doi.or 747 and 12:1255–63. https://doi.org/10.1172/JCl19159.
748 58 Eggermann T, Begemann M, Kurth I, Elbracht M. Contribution of GRB10 to the prenatal
749 phenotype in Silver-Russell syndrome? Lessons from 7p12 copy number varia 748 58 Eggermann T, Begemann M, Kurth I, Elbracht M.
749 phenotype in Silver-Russell syndrome? Lessons from 7p
750 *Genet* 2019;62:103671. https://doi.org/10.1016/j.ejmg
751 59 Wallace C, Smyth DJ, Maisuria-Armer M, Walker phenotype in Silver-Russell syndrome? Lessons from 7p12 copy number variations. Eur J Med

750 Genet 2019;62:103671. https://doi.org/10.1016/j.ejmg.2019.103671.

751 59 Wallace C, Smyth DJ, Maisuria-Armer M, Walker NM, Tod Phenotype in Silver-Russell syndrome? Lessons from 7p12 copy number variations. Let 3 Med

750 Genet 2019;62:103671. https://doi.org/10.1016/j.ejmg.2019.103671.

751 S9 Wallace C, Smyth DJ, Maisuria-Armer M, Walker NM, Tod The Center 2019;02:103671. https://doi.org/10.1010/j.ejing.2019.103671.

751 59 Wallace C, Smyth DJ, Maisuria-Armer M, Walker NM, Todd JA, C

1752 imprinted DLK1-MEG3 gene region on chromosome 14q32.2 alters sus

1753 diab imprinted DLK1-MEG3 gene region on chromosome 14q32.2 alters susceptibility to ty

diabetes. Nat Genet 2010;42:68–71. https://doi.org/10.1038/ng.493.

754 60 Tahara S, Tahara T, Horiguchi N, Okubo M, Terada T, Yoshida D, diabetes. Nat Genet 2010;42:68–71. https://doi.org/10.1038/ng.493.
754 60 Tahara S, Tahara T, Horiguchi N, Okubo M, Terada T, Yoshida D, et al. Lower LINE-1
755 methylation is associated with promoter hypermethylation and 753 diabetes. Nat Genet 2010, 42:68–71. https://doi.org/10.1038/ng.493.
754 60 Tahara S, Tahara T, Horiguchi N, Okubo M, Terada T, Yoshida D,
755 methylation is associated with promoter hypermethylation and disting
8 gast 754 60 Tahara 3, Tahara T, Horiguchi N, Okubo M, Terada T, Toshida D, et al. Lower LINE-1

755 methylation is associated with promoter hypermethylation and distinct molecular feature

756 gastric cancer. *Epigenomics* 2 $T56$ gastric cancer. *Epigenomics* 2019;11:1651–9. https://doi.org/10.2217/epi-2019-0091.

1 556 gastric cancer. Epigenomics 2019;11:1651–9. https://doi.org/10.2217/epi-2019-0091.

257 61 Ito Y, Roessler T, Ibrahim AEK, Rai 3, Vowler SL, Abu Amero 3, et al. Somatically acquired

258 https://doi.org/10.1093/hmg/ddn163.

260 62 Nye MD, King KE, Darrah TH, Maguire R, Jima DD, Huang Z, *et al.* Maternal The Hypomethylation of IGF2 in breast and colorectal cancer. Hum Mor Genet 2006;17:2003–43.

759 https://doi.org/10.1093/hmg/ddn163.

760 62 Nye MD, King KE, Darrah TH, Maguire R, Jima DD, Huang Z, *et al.* Maternal blood 760 62 Nye MD, King KE, Darrah TH, Mag

761 concentrations, DNA methylation of MI

762 and early growth in a multiethnic cohol

1763 https://doi.org/10.1093/eep/dvv009.

764 63 Li L, Zhang T, Qin X-S, Ge W, Ma F

765 (DEH 760 62 Nye MD, King K.C, Darrah TH, Maguie K, Jima DD, Huang 2, et al. Material blood lead

2761 concentrations, DNA methylation of MEG3 DMR regulating the DLK1/MEG3 imprinted doma

2762 and early growth in a multiethnic c 2762 and early growth in a multiethnic cohort. *Environ Epigenet* 2016;2:dvv009.

2763 https://doi.org/10.1093/eep/dvv009.

2764 63 Li L, Zhang T, Qin X-S, Ge W, Ma H-G, Sun L-L, et al. Exposure to diethylhexyl phthalate
 and early growth in a multiethnic cohort. *Environ Epigenet* 2016;2:dvv009.

763 https://doi.org/10.1093/eep/dvv009.

764 63 Li L, Zhang T, Qin X-S, Ge W, Ma H-G, Sun L-L, *et al.* Exposure to diethylhexyl phthalate

765 (764 63 Li L, Zhang T, Qin X-S, Ge W, Ma
765 (DEHP) results in a heritable modificat
766 oocytes. Mol Biol Rep 2014;41:1227–3
767 64 Kochmanski JJ, Marchlewicz EH,
768 Longitudinal Effects of Developmental
769 Hydroxymethy 764 63 Li L, Zhang T, Qin X-3, Ge W, Ma H-G, Sun L-E, et al. Exposure to diethyliexyl phthalate

765 (DEHP) results in a heritable modification of imprint genes DNA methylation in mouse

766 ocytes. *Mol Biol Rep* 2014;41: 766 oocytes. *Mol Biol Rep* 2014;41:1227–35. https://doi.org/10.1007/s11033-013-2967-7.
767 64 Kochmanski JJ, Marchlewicz EH, Cavalcante RG, Perera BPU, Sartor MA, Dolinoy
768 Longitudinal Effects of Developmental Bispheno 767 oocytes. Mol Biol Rep 2014;41:1227–35. https://doi.org/10.100//s11033-013-2307-7.
767 64 Kochmanski JJ, Marchlewicz EH, Cavalcante RG, Perera BPU, Sartor MA, Dolinoy I
1768 Hydroxymethylation at Imprinted Loci in Mouse Longitudinal Effects of Developmental Bisphenol A Exposure on Epigenome-Wide DNA

769 Hydroxymethylation at Imprinted Loci in Mouse Blood. *Environmental Health Perspectives*

770 n.d.;126:077006. https://doi.org/10.1289/E 1988 Hydroxymethylation at Imprinted Loci in Mouse Blood. Environmental Health Perspecti

1988 Hydroxymethylation at Imprinted Loci in Mouse Blood. Environmental Health Perspecti

1988 Malish JM, Jiang C, Bartolomei MS. Ep 1998 Hydroxymethylation at Imprinted Eochin Mouse Blood. *Environmental Health Perspectives*

1998 Ind.;126:077006. https://doi.org/10.1289/EHP3441.

1998 India Mersian Manuscope in Mouse Blood. Environmental Health Perspe man, and the proposed of the mandei of the mandei of the mann of the present of the present of the present of the mass of the presentations, genotypes, next of the presentations, genotypes, next of the present of the prese The Control Co 272 Dev Biol 2014;38:291–8. https://doi.org/10.1387/ijdb.140077mb.

773 66 Prasasya R, Grotheer KV, Siracusa LD, Bartolomei MS. Templ

2020;29:R107–16. https://doi.org/10.1093/hmg/ddaa133.

776 67 Doshi T, D'souza C, Vanag 779 x.
780 68 Schrott R, Greeson KW, King D, Symosko Crow KM, Easley CA, Murphy SK, Cannabis Ogata syndrome: clinical presentations, genotypes, models and mechanisms. Hum Mol Genet

2020;29:R107-16. https://doi.org/10.1093/hmg/ddaa133.

776 67 Doshi T, D'souza C, Vanage G. Aberrant DNA methylation at lgf2-H19 impr 2020; 2021, 2022, 2022, 2022, 2022, 2022, 2022, 2022, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 20

777 2022, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 2023, 20
 region in spermatozoa upon neonatal exposure to bisphenol A and its association with post

implantation loss. *Mol Biol Rep* 2013;40:4747–57. https://doi.org/10.1007/s11033-013-2571–

x.

Schrott R, Greeson KW, King D, Sym 778 implantation loss. *Mol Biol Rep* 2013;40:4747–57. https://doi.org/10.1007/s11033-013-257:
779 x.
780 68 Schrott R, Greeson KW, King D, Symosko Crow KM, Easley CA, Murphy SK. Cannabis
781 alters DNA methylation at mate 1778 Implantation loss. Mol Biol Rep 2013, 40:4747–57. https://doi.org/10.1007/s11033-013-2371-

779 x.

780 68 Schrott R, Greeson KW, King D, Symosko Crow KM, Easley CA, Murphy SK. Cannabis

1781 alters DNA methylation at 780 68
781 alt
782 ce
783 ht
784 69
785 fla 281 alters DNA methylation at maternally imprinted and autism candidate genes in spermatog

282 cells. Syst Biol Reprod Med 2022;68:357–69.

283 https://doi.org/10.1080/19396368.2022.2073292.

284 69 Soubry A, Hoyo C, Butt The Collis. Syst Biol Reprod Med 2022;68:357–69.

281 bttps://doi.org/10.1080/19396368.2022.2073292.

284 69 Soubry A, Hoyo C, Butt CM, Fieuws S, Price TM, Murphy SK, et al. Human exposure to

285 flame-retardants is assoc 782 cens. Syst Biol Reprod Med 2022,08:357–69.
783 https://doi.org/10.1080/19396368.2022.207
784 69 Soubry A, Hoyo C, Butt CM, Fieuws S, P
785 flame-retardants is associated with aberrant
786 *Environmental Epigenetics* 2 784 69 Soubry A, Hoyo C, Butt CM, Fieuws S, Price Tr
785 flame-retardants is associated with aberrant DNA n
786 *Environmental Epigenetics* 2017;3:dvx003. https://
787 70 Weinstein LS, Xie T, Zhang Q-H, Chen M. Stud
788 G 784 69 Soubry A, Hoyo C, Butt CM, Heaws S, Thee TM, Murphy SK, Et al. Human exposure to

185 flame-retardants is associated with aberrant DNA methylation at imprinted genes in sperm.

186 *Environmental Epigenetics* 2017;3 Fortian Frame-retardal Epigenetics 2017; 3: dvx003. https://doi.org/10.1093/eep/dvx003.

787 70 Weinstein LS, Xie T, Zhang Q-H, Chen M. Studies of the regulation and function of the

788 Gsα gene Gnas using gene targeting 787 70 Weinstein LS, Xie T, Zhang Q-H, Chen M. Studies of the regulation and function of Sax gene Gnas using gene targeting technology. *Pharmacol Ther* 2007;115:271–5 https://doi.org/10.1016/j.pharmthera.2007.03.013.
790 Gsα gene Gnas using gene targeting technology. *Pharmacol Ther 2007;115:271-91.*
789 https://doi.org/10.1016/j.pharmthera.2007.03.013.
790 71 Wang L, Chang S, Wang Z, Wang S, Huo J, Ding G, *et al.* Altered GNAS imprintin 789 Gsa gene Gnas using gene targeting technology. *Fharmacol Ther 2007*,115:271–91.
789 https://doi.org/10.1016/j.pharmthera.2007.03.013.
791 folic acid deficiency contributes to poor embryo development and may lead to ne 790 71 Wang L, Chang S, Wang Z, Wang S, Huo J, Ding
791 folic acid deficiency contributes to poor embryo deverted:
792 defects. Oncotarget 2017;8:110797–810. https://doi.
793 72 Hanna P, Francou B, Delemer B, Jüppner H, L 790 71 Wang E, Chang S, Wang S, Wang S, Huo S, Ding G, et al. Altered GNAS impiriting due to

791 folic acid deficiency contributes to poor embryo development and may lead to neural tube

792 defects. *Oncotarget* 2017;8:1 defects. Oncotarget 2017;8:110797–810. https://doi.org/10.18632/oncotarget.22731.

793 72 Hanna P, Francou B, Delemer B, Jüppner H, Linglart A. A Novel Familial PHP1B Variant

794 With Incomplete Loss of Methylation at GNA The United States. Oncotarget 2017;**3:110757–910.** https://doi.org/10.18632/oncotarget.22731.

793 72 Hanna P, Francou B, Delemer B, Jüppner H, Linglart A. A Novel Familial PHP1B Vai

794 With Incomplete Loss of Methylatio With Incomplete Loss of Methylation at GNAS-A/B and Enhanced Methylation at GNAS-AS2.

The Endocrinol Metab 2021;106:2779–87. https://doi.org/10.1210/clinem/dgab136.

Turan S, Bastepe M. The GNAS complex locus and human di With Incomplete Loss of Methylation at GNAS-A/B and Enhanced Methylation at GNAS-AS2. J
795 Clin Endocrinol Metab 2021;106:2779–87. https://doi.org/10.1210/clinem/dgab136.
796 73 Turan S, Bastepe M. The GNAS complex locus 795 Can Endocrinol Metab 2021,100.2779–87. https://doi.org/10.1210/clinem/dgab136.
796 73 Turan S, Bastepe M. The GNAS complex locus and human diseases associated v
797 of-function mutations or epimutations within this imp 797 of-function mutations or epimutations within this imprinted gene. Horm Res Paediatric 2013;80:10.1159/000355384. https://doi.org/10.1159/000355384. 798 2013;80:10.1159/000355384. https://doi.org/10.1159/000355384.

2799 74 Wroe Sr, Kelsey G, Skillier 3A, Bodie D, Ball Sr, Beechey Cv, et al. An imprinted

2800 transcript, antisense to Nesp, adds complexity to the cluster of imprinted genes at the

2802 75 Hayward BE, Kamiya M, Strain 801 Gnas locus. *Proc Natl Acad Sci U S A* 2000; 97:3342–6. https://doi.org/10.1073/pnas.97.7.3342
802 75 Hayward BE, Kamiya M, Strain L, Moran V, Campbell R, Hayashizaki Y, *et al.* The human
803 GNAS1 gene is imprinted a 802 T5 Hayward BE, Kamiya M, Strain L, Moran V, Campbell R, Hayashizaki Y, *et al.* The human
803 GNAS1 gene is imprinted and encodes distinct paternally and biallelically expressed G
804 proteins. *Proc Natl Acad Sci U S* 802 75 Hayward BE, Kamiya M, Strain L, Moran V, Campbell R, Hayashizaki Y, et al. The human 6NAS1 gene is imprinted and encodes distinct paternally and biallelically expressed G
804 proteins. *Proc Natl Acad Sci U S A* 199 804 proteins. *Proc Natl Acad Sci U S A* 1998;95:10038-43.
805 https://doi.org/10.1073/pnas.95.17.10038.
806 76 Desbuquois B, Carré N, Burnol A-F. Regulation of insulin and type 1 insulin-like g
807 factor signaling and ac 805 https://doi.org/10.1073/pnas.95.17.10038.
806 76 Desbuquois B, Carré N, Burnol A-F. Regulation of
807 factor signaling and action by the Grb10/14 and SH2B
808 2013;280:794–816. https://doi.org/10.1111/febs.1208
809 77 806 76 Desbuquois B, Carré N, Burnol A-F. Re

807 factor signaling and action by the Grb10/14

808 2013;280:794–816. https://doi.org/10.1111

809 77 Plasschaert RN, Bartolomei MS. Tissue

810 growth and neuronal commitmen Factor signaling and action by the Grb10/14 and SH2B1/B2 adaptor proteins. *FEBS J*

808 2013;280:794–816. https://doi.org/10.1111/febs.12080.

809 77 Plasschaert RN, Bartolomei MS. Tissue-specific regulation and function Factor signaling and action by the Grb10/14 and SH2B1/B2 adaptor proteins. FEBS 2013;280:794-816. https://doi.org/10.1111/febs.12080.
809 77 Plasschaert RN, Bartolomei MS. Tissue-specific regulation and function of Grb
810 2020

809 27 Plasschaert RN, Bartolomei MS. Tissue-specific regu

810 growth and neuronal commitment. *Proc Natl Acad Sci U S*

811 https://doi.org/10.1073/pnas.1411254111.

812 78 Hikichi T, Kohda T, Kaneko-Ishino T, Ishi 810 growth and neuronal commitment. *Proc Natl Acad Sci U S A* 2015;112:6841–7.
811 https://doi.org/10.1073/pnas.1411254111.
812 78 Hikichi T, Kohda T, Kaneko-Ishino T, Ishino F. Imprinting regulation of the murine
813 Meg 811 https://doi.org/10.1073/pnas.1411254111.
812 78 Hikichi T, Kohda T, Kaneko-Ishino T, Ishino F. Imprinting regulation of the
813 Meg1/Grb10 and human GRB10 genes; roles of brain-specific promoters and m
814 CTCF-binding 812 78 Hikichi T, Kohda T, Kaneko-Ishino T, Isl
813 Meg1/Grb10 and human GRB10 genes; role:
814 CTCF-binding sites. Nucleic Acids Res 2003;3
815 79 Luo L, Jiang W, Liu H, Bu J, Tang P, Du
816 ER stress-induced steatosis in Meg1/Grb10 and human GRB10 genes; roles of brain-specific promoters and mouse-sp

814 CTCF-binding sites. Nucleic Acids Res 2003;31:1398–406. https://doi.org/10.1093/nar/g

815 79 Luo L, Jiang W, Liu H, Bu J, Tang P, Du C, 814 CTCF-binding sites. *Nucleic Acids Res* 2003;31:1398-406. https://doi.org/10.1093/nar/gkg232
815 79 Luo L, Jiang W, Liu H, Bu J, Tang P, Du C, et al. De-silencing Grb10 contributes to acute
816 ER stress-induced steato 815 79 Luo L, Jiang W, Liu H, Bu J, Tang P, Du C, et al. De-silencing Grb 10 contributes to acute
816 ER stress-induced steatosis in mouse liver. *J Mol Endocrinol* 2018;60:285–97.
817 https://doi.org/10.1530/JME-18-0018.
 816 ER stress-induced steatosis in mouse liver. *J Mol Endocrinol* 2018;60:285–97.
817 https://doi.org/10.1530/JME-18-0018.
818 80 Blagitko N, Mergenthaler S, Schulz U, Wollmann HA, Craigen W, Eggermann T, *et al.*
819 Hum 817 https://doi.org/10.1530/JME-18-0018.
818 80 Blagitko N, Mergenthaler S, Schulz U, Wollmann HA, Craigen W, Eggerm
819 Human GRB10 is imprinted and expressed from the paternal and maternal all
820 tissue- and isoform-spe 818 80 Blagitko N, Mergenthaler S, Schul
819 Human GRB10 is imprinted and express
820 tissue- and isoform-specific fashion. Hu
821 https://doi.org/10.1093/hmg/9.11.158
822 81 Bakulski KM, Feinberg JI, Andrews
823 methylati 819 Blagitto N, Mergentinaer 3, Schulz O, Wollmann HA, Craigen W, Eggermann T, et al.
819 Human GRB10 is imprinted and expressed from the paternal and maternal allele in a highl
820 tissue- and isoform-specific fashion. Hu 820 tissue- and isoform-specific fashion. Human Molecular Genetics 2000;9:1587-95.
821 https://doi.org/10.1093/hmg/9.11.1587.
822 81 Bakulski KM, Feinberg JI, Andrews SV, Yang J, Brown S, L. McKenney S, et al. DNA
823 meth 820 tissue- and isoform-specific fashion. Human Molecular Genetics 2000, 3:1587–95.
821 https://doi.org/10.1093/hmg/9.11.1587.
823 methylation of cord blood cell types: Applications for mixed cell birth studies. *Epig*
824 822 81 Bakulski KM, Feinberg JI, Andrews S
823 methylation of cord blood cell types: App
824 2016;11:354–62. https://doi.org/10.1080
825 82 Armand EJ, Li J, Xie F, Luo C, Mukam
826 Transcriptomes and Epigenomes. Neuron
827 823 Bakulski KM, Feliberg J., Andrews SV, Tang J., Brown 3, L. McKenney 3, et al. DNA
823 methylation of cord blood cell types: Applications for mixed cell birth studies. *Epigeneti*
824 2016;11:354–62. https://doi.org/10. S23 methylation of cord blood cell types: Applications for mixed cell birth studies. Epigenetics

824 2016;11:354–62. https://doi.org/10.1080/15592294.2016.1161875.

825 82 Armand EJ, Li J, Xie F, Luo C, Mukamel EA. Single 825 82 Armand EJ, Li J, Xie F, Luo C, Mukamel EA. Single-Cell Sequencii
826 Transcriptomes and Epigenomes. *Neuron* 2021;109:11–26.
827 https://doi.org/10.1016/j.neuron.2020.12.010.
828 83 Campbell KA, Colacino JA, Park SK 826 Transcriptomes and Epigenomes. Neuron 2021;109:11–26.
827 https://doi.org/10.1016/j.neuron.2020.12.010.
828 83 Campbell KA, Colacino JA, Park SK, Bakulski KM. Cell types in environmental er
829 studies: Biological and 827 Transcriptomes and Epigenomes. Neuron 2021,109.11–20.
828 83 Campbell KA, Colacino JA, Park SK, Bakulski KM. Cell t
829 studies: Biological and epidemiological frameworks. Curr En
830 https://doi.org/10.1007/s40572-020 828 83 Campbell KA, Colacino JA, Park SK, Bakuls
829 studies: Biological and epidemiological frameworchttps://doi.org/10.1007/s40572-020-00287-0.
831 829 studies: Biological and epidemiological frameworks. *Curr Environ Health Rep* 2020;**7**:185–97.
830 https://doi.org/10.1007/s40572-020-00287-0.
831 829 studies: Biological and epidemiological frameworks. Curr Environ Health Rep 2020;7:185–97.
830 https://doi.org/10.1007/s40572-020-00287-0.
831 831

