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Incorporation of an Epigenetic Evaluation into Safety Assessment: What we First Need to Know

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Abstract

The rapidly evolving field of epigenetic regulation of gene expression is having an impact across the spectrum of biomedical research. Toxicologists have embraced this area as evidenced by their increasing focus on discerning potential epigenetic mechanisms underlying mechanisms by which chemical and physical agents might cause toxicity. It is not surprising that an interest in epigenetic mechanisms of toxicity would lead to a desire to incorporate an epigenetic component into safety assessment. However, premature movement in this direction carries the risk of imposing more confusion than light. This commentary provides an overview of epigenetics, with an emphasis on how the various epigenetic parameters are integrated, as a basis for understanding the complexity behind the desire to include epigenetic evaluations in safety evaluations. Basically, we have much more to learn before turning the goal into a reality. However, considerable progress has been made with regard to using epigenetic profiles as signatures of xenobiotic exposure.

Keywords

DNA Methylation; Epigenetics; Histone Code; Micro RNA; Non-Coding RNA; Safety Assessment

1. Introduction

The term “epigenetics” stems from the Greek word “epi,” which means “on” or “above” and refers to mechanisms superimposed on DNA sequence that regulate gene expression. A brief, subjective history of epigenetics focused on DNA methylation (i.e., a methyl group at the 5-position of DNA cytosine) will set the stage for this “Opinion.” In 1942, Waddington [1] was the first person to talk about epigenetics when he used the term “epigenotype” to refer to the developmental processes between genotype and phenotype. Two seminal papers

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published in 1975 were the first to suggest that sequence-specific methylases in eukaryotes could add a methyl group at the 5-position of DNA-cytosine and that this DNA methylation could affect the binding of regulatory proteins which control gene activity during development [2, 3]. Supporting evidence for this hypothesis was provided in 1979 when it was shown that new phenotypes could be induced in mammalian cells by treatment with 5-azacytidine [4]. In 1984 it was shown that 5-azacytidine acts as a non-competitive inhibitor of DNA (cytosine-5)-methyltransferase and can cause hypomethylation of DNA in mammalian cells [5]. The ability of 5-azacytidine to act, following its incorporation into DNA, to inhibit DNA methyltransferase resulting in new cytosine methylation patterns (hypomethylation) leading to altered gene expression and differentiation was first reviewed in a comprehensive fashion in 1985 [6]. Thus, as it has become apparent that epigenetic mechanisms underlie differentiation and specify the phenotype of cells it is not unexpected to see that the number of publications and interest in this area of science have increased at a stunning rate in recent years. This is certainly true in Toxicology where there is a growing recognition that epigenetic alterations might play key roles in mechanisms underlying the ability of chemical and physical agents to cause toxicity. This has lead, logically, to an interest in incorporating an epigenetic evaluation (including but not limited to DNA methylation) into safety assessment, e.g., does the chemical of interest have the capacity to cause an epigenetic change(s)? At first glance the proposal might appear to be reasonable. A workshop on epigenetics and safety assessment was held in 2009 and at its conclusion the consensus was that much needs to be learned prior to being able to rationally incorporate an epigenetic evaluation into safety assessment [7]. The author's current opinion is that, while our knowledge of epigenetics has expanded at an extraordinary pace over the past eight years and continued exploratory research is highly desirable, the state of the science remains such that we are not ready to routinely add epigenetic evaluations into safety assessment (or risk assessment). Indeed, to do so at this time would be counterproductive.

2. Epigenetic Parameters

There are four broad categories of epigenetic parameters which combine to regulate gene expression in mammals:

2.1 The first is modifications at the 5-position of DNA cytosine.

This includes 5-methylcytosine (DNA methylation, 5mC) an epigenetic mark that is often referred to as the 5th DNA-base. The DNA methylation field has expanded markedly as a result of the discoveries that 5mC can be oxidized by the ten eleven translocation protein (TET) family of dioxygenases to successively form 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fM) and 5-carboxycytosine (5cM) [8]. 5cM can be excised from DNA by a base excision repair glycosylase [9]. Thus, the sequential TET-mediated oxidation of 5mC to 5hC to 5fC to 5cM represents a pathway for demethylation of DNA.

However, the three 5mC oxidation products do not simply represent steps towards demethylation. On the contrary, evidence is accumulating indicating that they have functional epigenetic roles distinct from 5mC [10]. For example, proteins which bind to 5hC or 5fC have been identified, suggesting they play a role in regulating transcription [11].

Gene and site-specific aspects of the distribution of 5hC in DNA has been suggested to contribute to the epigenetic control of hepatic genes [12] and a reciprocal relationship has been observed between 5mC and 5hC in specific mouse liver genes in response to treatment with phenobarbital [13].

An exciting new development in the area of DNA methylation is discovery that DNA methylation on N⁶-Adenine occurs in mammalian embryonic cells and is a component of the epigenetic regulation repertoire [14].

2.2 The second is the histone code.

The DNA of eukaryotes is packaged into chromatin that has a repeating unit referred to as a nucleosome which consists of 147 base pairs of DNA wrapped around an octamer consisting of four histone proteins (H2A, H2B, H3 and H4) [15]. A variety of posttranslational modifications, typically referred to as the histone code, of the histone proteins can alter the structure and function of the nucleosome and, thus, affect transcription [15,16]. We typically think of nine classes of modifications of histone proteins (acetylation, methylation of lysines, methylation of arginines, phosphorylation, ubiquitylation, sumoylation, ADP ribosylation, deamination and proline isomerization), each enzymatic can be carried out by multiple isoforms, and there are enzymatic mechanisms for their removal [17]. Additionally, new histone marks are expected to be discovered, e.g., a new histone modification has been discerned recently, lysine 2-hydroxyisobutyrylation [18].

2.3 The third consists of non-coding RNAs.

A variety of non-coding (i.e., non-protein coding) RNAs play key roles in epigenetic regulation of gene expression. These include, but are not limited to microRNAs (miRNAs) which comprise a class of RNAs that are approximately 18–25 nucleotides in length and regulate gene expression posttranscriptionally [19] and long intervening noncoding RNAs (lincRNAs) that are usually >200 nucleotides in length [20] and can act as scaffolds to recruit chromatin modifying enzymes to specific regions of the genome [21]. In addition to the ability of non-coding RNAs to affect chromatin structure they can be involved in directing DNA methyltransferases to particular sites on DNA [22].

2.4 The fourth category is the newest aspect of epigenetics, the epitranscriptome.

More than 100 chemically distinct modifications of ribonucleosides have been detected [23]. In particular, with regard to epigenetics, emphasis is being placed on the methylation of mRNA-adenosine to form N⁶ methyladenosine (m⁶ A), which is reversible and represents a new aspect of post-transcriptional gene regulation in eukaryotes. Proteins that specifically recognize m⁶A have been identified and this mark has been shown to affect mRNA splicing, maturation and translation [24]. Other mRNA modifications, including N¹-methyladenosine (m¹A), 5-methylcytosine (m⁵C) and pseudouridine, together with m⁶A form the epitranscriptome and collectively code a new layer of information that controls protein synthesis [24, 25].

It is important to understand that the epigenetic parameters interact with each other and there is likely a good deal of redundancy/buffering in the systems. For example, as noted above,

lincRNAs can act as scaffolds to recruit chromatin modifying enzymes to specific regions of the genome [21], non-coding RNAs can affect chromatin structure and be involved in directing DNA methyltransferases to particular sites on DNA [22]. Additionally, miRNAs can affect methylation by targeting mRNAs which code for methyltransferases [26].

3. What One Must Consider Prior to Incorporating an Epigenetic Evaluation into Safety Assessment

In light of the discussion presented above, it is quite obvious that incorporation of an epigenetic evaluation into safety assessment presents a very difficult, multifaceted problem. This starts with the need to ask is there an issue, are there indications that chemical-induced adverse effects in humans might really be a problem? There are suggestions that the answer might be “yes” [27, 28]. Thus, there is an interest in incorporating an epigenetic evaluation into safety assessment. However, elucidating epigenetic determinants of human disease is a major challenge for biomedical research [29]. To succeed in identifying epigenetic mechanisms leading to complex phenotypes and disease, researchers must integrate the various animal models, human clinical approaches, and human population approaches while paying attention to life-stage sensitivity, to generate effective prescriptions for human health evaluation and disease prevention [30]. Clearly, this is not an easy task.

In order to plan for laboratory research one must address a number of basic issues. Decisions must be made regarding what model system(s) will be employed, e.g., rodents, zebra fish, *C. elegans*, *drosophila*, which model compounds will be employed, just what it is that is being looked for, e.g., what is the apical endpoint(s), and how might results be translated to humans? Substantial consideration has to be given to determining which epigenetic parameter(s) will be evaluated and what methodology(ies) will be used. There is considerable interest in measuring 5mC. However, the basic techniques involving the use of enzymatic approaches or bisulfite sequencing to distinguish 5mC from DNA-cytosine is not capable of differentiating 5mC from 5hC [31, 32]. Thus, the DNA methylation data in the literature based on the use of enzymatic approaches or bisulfite sequencing are not correct. There are affinity-based techniques for enrichment of 5hC marked DNA for downstream analysis [33] and there is a particularly attractive, high throughput method for evaluating 5hC which involves immunoprecipitation and semiconductor sequencing [34]. An example of the importance of distinguishing between 5mC and 5hC is provided in a recent study in which the effect of phenobarbital (PB) treatment of B6C3F1 mice revealed dynamic, reciprocal changes in 5mC and 5hC in target genes [13]. Had the author used the standard bisulfite-based technique this would not have been detected. Subsequent studies on expression and methylation status of the gene which encodes *Cyp2b10*, a PB-target gene, demonstrated that the promoter region was hypomethylated concomitant with transcriptional activation and analysis of active and repressive histone marks revealed an epigenetic switch [35]. This demonstrates the power of examining modifications at the 5-position of DNA cytosine in combination with an evaluation of aspects of the histone code.

When planning to evaluate epigenetic parameters the extent of normal variability needs to be ascertained. For example, In the human brain DNA methylome (pattern of DNA

methylation), with the exception of cerebellum, DNA methylation patterns are more homogeneous between different brain regions from the same individual than they are for a single brain region between different individuals [36]. Additionally, in animal experimentation one must keep in mind the fact epigenetic parameters likely play a key role in regulating diurnal variations in gene expression, and that tissues are composed of multiple cell types and when a sample is evaluated the results represent an average among the different cells. Therefore, if chemical treatment changes the balance of cell types, e.g., if it causes an infiltration of inflammatory cells, one will see a change in epigenetic parameters due, perhaps in part, to the new mixture of cells sampled rather than solely due to changes in the expected cell types in the particular tissue. Thus, knowledge of the histology is a must. Epigenetic patterns change with age, e.g., epigenetic drift occurs in aging identical twins [37]. Therefore, provision for age-matched controls has to be included in the experimental protocol. Diet can also affect epigenetic parameters, e.g., dietary changes lead to increased epigenetic variation in isogenic mice [38], and we can anticipate that culture medium will affect the epigenetic status of cells in culture.

A key component of all toxicology studies, is giving particular consideration to interpretation of results regarding making the distinction between a change, which could be adaptive, as compared to what might appropriately be deemed an adverse effect. At the present time, it is especially difficult to make the linkage between an apical adverse endpoint and a particular alteration of an epigenetic parameter(s). Additionally, epigenetic alterations might be reversible, neutral (i.e. result in no effect) or, even beneficial. The genome of the viable yellow agouti (A^{vy}) mouse contains a metastable Agouti gene due to insertion of an intracisternal A particle (IAP) retrotransposon containing a cryptic promoter at its proximal end. The mutant exhibits decreased methylation of the Agouti locus leading to deeper penetrance of a yellow coat color and an obese phenotype. Maternal dietary supplementation during pregnancy with either methyl donating substances, i.e. folic acid, vitamin B12, choline and betaine [39] or genistein [40], a phytoestrogen present in soy products results in increased CpG methylation of the IAP promoter leading to more offspring having a wild type coat color along with a decreased prevalence of obesity. The points that must be considered prior to incorporating an epigenetic evaluation into safety assessment are depicted in Figure 1

4. Conclusion and Perspectives

There is interest in incorporating an epigenetic evaluation into safety assessment [41, 42]. However, in light of the discussion presented above, it is apparent that we are not at the point where epigenetic evaluations can be routinely incorporated into safety assessments. There are too many knowledge gaps concerning the basic information that is required. To be clear, I am not saying that one must know “everything” in order to move forward, and I do not want to encourage paralysis by obsessive analysis. However, knowledge gaps need to be filled, and rationales developed to provide an appropriate level of attention to the points outlined in Figure 1. For example, which model system(s) should be employed, which epigenetic parameters should be evaluated, what region(s) of the genome should be evaluated, e.g., particular genes or perhaps the clusters of enhancers, known as super-enhancers, that specify cell type identity and function [43] and what constitutes a change v.

and adverse effect? Of course, as in all toxicology studies, understanding normal variability in experimental models and in humans where there is substantial inter-individual epigenetic variation [44], dose-response and time-response relationships, and, importantly, setting rational criteria for the highest dose/concentration used are fundamental issues to consider. One aspect that is holding back the ability to effectively incorporate an epigenetic evaluation into safety assessment is a relative lack of research designed to provide the type of information (e.g., dose-response and time-response relationships, connecting an epigenetic alteration(s) with an apical endpoint) required for these types of toxicology studies.

When considering hypothesis driven research, epigenetic regulation of gene expression is a valuable approach to understand mechanisms (35, 45, 46, 47). Furthermore, with regard to safety assessment, one area where real progress is being made is in identifying epigenetic profiles that provide signatures of xenobiotic exposure [48].

References

Papers of particular interest have been highlighted as: * of special interest

- [1] *. Waddington CH The epigenotype. *Endeavour* 1 (1942) 18–20.
of special interest
- [2] *. Holliday R, Pugh JE DNA modification mechanisms and gene activity during development. *Science* 187 (1975) 226–232.
of special interest
- [PubMed: 1111098]
- [3] *. Riggs AD X inactivation, differentiation, and DNA methylation. *Cytogenetics and Cell Genetics* 14 (1975) 9–25.
of special interest
- [PubMed: 1093816]
- [4]. Taylor SM, Jones PA Multiple new phenotypes induced in 10T ½ and 3T3 cells treated with 5-azacytidine. *Cell* 17 (1979) 771–779. [PubMed: 90553]
- [5]. Lu LJW, Randerath K Longterm instability and molecular mechanism of 5-azacytidine-induced DNA hypomethylation in normal and neoplastic tissue. *Mol. Pharmacol* 26 (1984) 594–603. [PubMed: 6208475]
- [6]. Jones PA Effects of 5-azacytidine and its 2-deoxyderivative on cell differentiation and DNA methylation. *Pharmac. Ther* 28 (1985) 17–27.
- [7]. Goodman JI, Auhustine KA, Cunningham ML, Dixon D, Dragan YP, Falls JG, Rasoulpour RJ, Sills RC, Storer RD, Wolf DC, Pettit SD What do we need to know prior to thinking about incorporating an epigenetic evaluation into safety assessments. *Toxicological Sci.* 116 (2010) 375–381.
- [8]. Kriaucionis S, Tahiliani M Expanding the epigenetic landscape: Novel modifications of cytosine in genomic DNA. *Cold Spring Harbor Perspectives in Biology* (2014) 6:a018630. [PubMed: 25274704]
- [9]. Bhutani N, Burns DM, Blau HM DNA demethylation dynamics. *Cell* 146 (2011) 866–872. [PubMed: 21925312]
- [10]. Song CX, He C Potential functional roles of DNA demethylation intermediates. *Trends in Biochemical Sciences* 38 (2013) 480–484. [PubMed: 23932479]
- [11]. Lurlaro M, Ficuz G, Oxley D, Raiber EA, Bachman M, Booth MJ, Andrews S, Balasubramanian S, Reik W *Genome Biology* 14 (2013) 14:R119. [PubMed: 24156278]

- [12]. Ivanov M, Kals M, Lauschke V, Barragan I, Ewels P, Kaller M, Axelsson T, Lehtio J, Milani L, Ingelman-Sundberg M Single base resolution analysis of 5-hydroxymethylcytosine in 188 human genes: Implications for hepatic gene expression. *Nuc. Acids. Res* 44 (2016) 6756–6769.
- [13]. Thomson JP, Hunter JM, Lempiainen H, Muller A, Terranova R, Moggs JG, Meehan RR Dynamic changes in 5hydroxymethylation signatures underpin early and late events in drug exposed liver. *Nuc. Acids Res* 41 (2013) 5639–5654.
- [14] *. Wu TP, Seetin WG, Yongquan Y, Zhu S, Lin K, Liu Y, Byrum SD, Mackintosh SG, Zhong M, Tackett A, Wang G, Hon LS, Fang G, Swenberg JA, Xiao AZ DNA methylation on N⁶-adenine in mammalian embryonic stem cells. *Nature* 532 (2016) 3239–345.
- of special interest
- [15]. Karl R, Chung HR, Lasserre J, Vlahovicek K, Vingron M Histone modification levels are predictive for gene expression. *PNAS* 107 (2010) 2926–2931. [PubMed: 20133639]
- [16]. Bemstein BE, Meissner A, Lander ES The mammalian epigenome. *Cell* 128 (2007) 669–681. [PubMed: 17320505]
- [17]. Kouzarides T Chromatin modifications and their function. *Cell* 128 (2007) 693–705. [PubMed: 17320507]
- [18]. Dai L, Peng C, Montellier E, Lu Z, Chen Y, Ishii H, Debernardi A, Buchou T, Rousseaux S, Jin F, Sabari BR, Deng Z, Allis CD, Ren B, Khochbin S, Zhao Y *Nature Chem. Biol* 10 (2014) 365–373. [PubMed: 24681537]
- [19]. Sato F, Tsuchiya S, Meltzer SJ, Shimizu K MicroRNAs and epigenetics. *FEBS Journal* 278 (2011) 1598–1609. [PubMed: 21395977]
- [20]. Ulitsky I, Bartel DP LincRNAs: Genomics, evolution, and mechanisms. *Cell* 154 (2013) 26–46. [PubMed: 23827673]
- [21]. Holoch D, Moazed D RNA-mediated epigenetic regulation of gene expression. *Nature Rev. Genetics* 16 (2015) 71–84. [PubMed: 25554358]
- [22]. Mattick JS, Amaral PP, Dinger ME, Mercer TR, Mehler MF RNA regulation of epigenetic processes. *BioEssays* 31 (2009) 51–59. [PubMed: 19154003]
- [23]. Machnicka MA, Milanowska K, Oglou OO, Purta E, Kurkowska M, Olchowik A, Januszewski W, Kalinowski S, Dunin-Horkawicz S, Rother KM, Helm M, Bujnicki JM, Grosjean H MDOMICS: A database of RNA modification pathways-2013 update. *Nucleic Acides Res.* 41 (2013) D262–D267.
- [24] *. Zhao BS, Roundtree IA, He C Post-transcriptional gene regulation by mRNA modifications. *Nature Rev. Mol. Cell Bio* 18 (2017) 31–42.
- of special interest
- [PubMed: 27808276]
- [25] *. Helm M, Motorin Y Detecting RNA modifications in the epitranscriptome: Predict and validate. *Nature Rev. Genetics* 18 (2017) 275–290.
- of special interest
- [PubMed: 28216634]
- [26]. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Kay Huebner CM Croce. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *PNAS* 104 (2007) 15805–15810. [PubMed: 17890317]
- [27]. Jirtle RL, Skinner MK *Nature Rev. Environmental epigenomics and disease susceptibility.* *Genetics* 8 (2007) 253–262. [PubMed: 17363974]
- [28]. Waterland RA, Michels KB Epigenetic epidemiology of the developmental origins hypothesis. *Ann. Rev. Nutrition* 27 (2007) 363–388. [PubMed: 17465856]
- [29]. Rakyen VK, Down TA, Balding DJ, Beck S Epigenome-wide association studies for common human diseases. *Nature Rev. Genetics* 12 (2011) 529–541. [PubMed: 21747404]

- [30]. Rozek LS, Dolinoy DC, Sartor MA, Omen GS Ann. Rev. Public Health 35 (2014) 105–122. [PubMed: 24641556]
- [31]. Nestor C, Ruzov A, Meehan RR, Dunican DS Enzymatic approaches and bisulfite sequencing cannot distinguish between 5-methylcytosine and 5-hydroxymethylcytosine in DNA. BioTechniques 48 (2010) 317–319. [PubMed: 20569209]
- [32]. Huang Y, Pastor WA, Shen Y, Tahiliani M, Liu DR, Rao A The behavior of 5-hydroxymethylcytosine in bisulfite sequencing. PLoS ONE 5 (2010) e8888.
- [33]. Thompson JP, Hunter JM, Nestor CM, Dunican DS, Terranova R, Moggs JG, Meehan RR Comparative analysis of affinity-based 5-hydroxymethylation enrichment techniques. Nuc. Acids Res 42 (2013) No. 22, e206.
- [34]. Thompson JP, Fawkes A, Ottaviano R, Hunter JM, Shukla R, Mjoseng HK, Clark R, Coutts A, Murphy L, Meehan RR DNA immunoprecipitation semiconductor sequencing (DIP-SC-seq) as a rapid method to generate genome wide epigenetic signatures. Scientific Reports (2015) DOI: 10.1038/srepo9778.
- [35] *. Lempiainen H, Muller A, Brasa S, Teo SS, Roloff TC, Morawiec L, Zmurovic N, Vicart A, Funhoff E, Couttet P, Schubeler D, Grenet O, Marlowe J, Moggs J, Terranova R Phenobarbital mediates an epigenetic switch at the constitutive androstane receptor (CAR) target gene Cyp2b10 in the liver of B6C3F1 mice. PLoS ONE 6 (2011) Issue 3, e18216
of special interest
- [36]. Robert S Illingworth^{1,*}, Ulrike Gruenewald-Schneider¹, Dina De Sousa¹, Shaun Webb C Merusi ARW Kerr KD James C Smith R Walker R Andrews AP Bird. Inter-individual variability contrasts with regional homogeneity in the human brain DNA methylome. Nuc. Acids Res 43 (2015) 732–744.
- [37]. Martin GM Epigenetic drift in aging identical twins. PNAS 102 (2005) 14013–14014.
- [38]. Li CCY, Copley JE, Cowley MJ, Preiss T, Martin DIK, Suter CM A sustained dietary change increases epigenetic variation in isogenic mice. PLoS Genetics. 7 (2011) e1001380. [PubMed: 21541011]
- [39]. Waterland RA, Jirtle RL Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Cell Mol Biol. 23 (203) 5293–5300.
- [40]. Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL Maternal genistein alters coat color and protects A^{vy} mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect. 114, (2006) 567–572. [PubMed: 16581547]
- [41]. van Ravenzwaay B, Kleijnans J, Vrijhof H Editorial - Epigenetics and chemical safety. Mut. Res./Genetic Toxicol. Environ. Mutagenesis 764–765 (2014) 1–2.
- [42]. Miousse IR, Currie R, Datta K, Ellinger-Ziegelbauer H, French JE, Harrill AH, Koturbash I, Lawton M, Mann D, Meehan RR, Moggs JG, O'Lone R, Rasoulpour RJ, Reijo Pera RA, Thompson Karol Importance of investigating epigenetic alterations for industry and regulators: An appraisal of current efforts by the Health and Environmental Sciences Institute. Toxicology 335 (2015) 11–19. [PubMed: 26134581]
- [43]. Heinz S, Romanoski CE, Benner C, Glass CK The selection and function of cell type specific enhancers. Nature Rev. Mol. Cell Biol 16 (2015) 144–154. [PubMed: 25650801]
- [44]. Bock C, Walter J, Paulsen M, Lengauer T Inter-individual variation of DNA methylation and its implications for large-scale epigenome mapping. Nuc. Acids Res 36 (2008) e55
- [45] *. Phillips JM, Burgoon LD, Goodman JI Phenobarbital elicits unique, early changes in the expression of hepatic genes that affect critical pathways in tumor-prone B6C3F1 mice. Toxicol. Sci 109 (2009) 193–205.
of special interest
- [PubMed: 19270015]
- [46] *. Phillips JM, Burgoon LD, Goodman JI The constitutive active/androstane receptor facilitates unique phenobarbital-induced expression changes of genes involved in key pathways in precancerous liver and liver tumors. Toxicol. Sci 110 (2009) 319–333.
of special interest

[PubMed: 19482888]

- [47]. Lempiinen H, Couttet P, Bolognani F, Müller A, Dubost V, Luisier R, Rio Espinola A, Vitry V, Unterberger EB, Thomson JP, Treindl F, Metzger U, Wrzodek C, Hahne F, Zollinger T, Brasa S, Kalteis M, Marcellin M, Giudicelli F, Braeuning A, Morawiec L, Zamurovic N, Langle U, Scheer N, Schübeler D, Goodman J, Chibout SD, Marlowe J, Theil D, Heard DJ, Grenet O, Zell A, Templin MF, Meehan RR, Wolf RC, Elcombe CR, Schwarz M, Moulin P, Terranova R, Moggs JG Identification of Dlk1-Dio3 imprinted gene cluster noncoding RNAs as novel candidate biomarkers for liver tumor promotion. *Toxicol. Sci* 131 (2013) 375–386. [PubMed: 23091169]
- [48] *. Thompson JP, Moggs JG, Wolf CR, Meehan RR Epigenetic profiles as defined signatures of xenobiotic exposure. *Mut. Res./Genetic Toxicol. Environ. Mutagenesis* 7864–765 (2014) 3–9. of special interest

Highlights

- An integrated overview of epigenetics
- Points to consider prior to incorporating an epigenetic evaluation into safety assessment

Figure 1:
The points that must be considered prior to incorporating an epigenetic evaluation into safety assessment are depicted in Figure 1.