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The interface of epigenetics and toxicology in product safety assessment

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Abstract

Recent advancements in science and technology have exponentially increased our understanding of epigenetics, and it is important to examine these advancements in the context of potential benefits to safety assessments of chemical products. Product safety assessment is an essential component of product development and is applied to both "new" and "existing" chemical products. The current human health safety assessment paradigm consists of characterizing dose responses for adverse apical outcomes across a multitude of toxicology studies to identify a point of departure (POD) and does not typically include the characterization of genomic or epigenomic changes. Given the fundamental mechanistic intersection of molecular (e.g. epigenetic) and apical changes, it would be anticipated that an adverse apical outcome identified in a regulatory guideline study would be preceded by an epigenetic change. While epigenetics is not currently being utilized in regulatory risk assessments to date, there is promising potential to improve future risk assessment strategies by incorporating epigenetic endpoints. For example, epigenetic endpoints could be used as biomarkers of traditional adverse apical effects or incorporated into adverse outcome pathways (AOPs) or benchmark dose (BMD) approaches to identify a POD. In this manuscript, the role of epigenomic changes in the context of human product safety assessment is examined.

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1. Introduction

The development of new, innovative products is a complex process that requires large expenditures in cost and time. "Products" is a general term referring to both natural and synthetic chemicals and plant protection products (PPPs). Safety assessment is an essential component of product development and is applied to both "new" and "existing" chemical products. The driver for toxicity testing programs and mode of action (MoA) assessment for chemical products depends on the intended use. Advancements in science and technology continually increase our understanding of biology, and it is important to examine these advancements in the context of potential benefits to safety assessments of chemical products. Epigenetics is a rapidly evolving field that holds promise for improving current safety assessment paradigms for chemical products. To gain an understanding of the potential benefit of incorporating epigenetics into regulatory toxicity testing, it is important to put it into perspective with the current product safety assessment model.

Product safety assessment is based upon the core tenant of risk assessment, where risk is a function of the intrinsic hazards of a product in combination with potential human exposure. The ultimate goal of the risk assessment process is to ensure that potential human exposure via different scenarios does not exceed reference values (e.g., chronic reference dose), which are derived from applying uncertainty factors to points of departure (PODs). A POD can be defined as the highest dose level on the dose response curve producing no effect, or the dose level producing a predetermined response level associated with adversity. PODs can be derived from guideline mammalian toxicity studies, which range in complexity from acute toxicity assessments to multi-generational reproduction and carcinogenicity studies.

The Organization for Economic Cooperation and Development (OECD) provides comprehensive test guidelines for registration of products. These studies include *in vitro* and *in vivo* testing strategies to assess multiple endpoints and, for *in vivo* testing, generate dose-response relationships for observed adverse effects. The potential hazards that may be detected include specific target organ toxicity, immunotoxicity, genotoxicity, carcinogenicity, neurotoxicity, developmental toxicity, and reproductive toxicity. The incidence and/or severity of these hazards is assessed by measuring numerous apical end points for determination of adversity. Taking into account all of the changes observed in a

particular study, a Lowest-Observed-Adverse-Effect-Level (LOAEL) and No-Observed-Adverse-Effect-Level (NOAEL) are identified. Traditionally, LOAELs and NOAELs across all of the studies performed on a molecule are taken into a weight-of-evidence approach to identify the previously described POD and perform a risk assessment. Alternatively, a POD may be determined using the benchmark dose (BMD) approach which uses all the data available across the dose response curve to identify a dose level resulting in a predetermined effect level. The POD is used to calculate the chronic reference dose (cRfD) or acceptable daily intake (ADI) for a molecule by dividing the POD by relevant uncertainty factors, such as 10× for intraspecies and 10× for interspecies differences. For the purposes of cancer risk assessment, generally speaking the typical methods employed depend upon whether the MoA for a given tumor is well-defined and consistent with current biological understanding. For example, for a prototypical constitutive androstane receptor (CAR)-mediated rodent liver tumor inducer, the dose-response data may be mathematically modeled and fit to a curve where the lower 95% confidence limit on a dose associated with a 10% increased tumor or relevant nontumor response (LED10) is identified. This derived dose can then be used as a POD for risk assessment and margin-of-safety evaluations. While epigenomic endpoints have not been utilized in product safety assessment regulatory decisions to date, we believe opportunities are available to harness epigenetics to build upon current product safety assessment paradigms.

Biological systems are built upon an interconnected hierarchy: molecule to cell to tissue to organ to organism (and to the population level in ecological assessments). Molecular processes, such as gene expression changes, are the foundation of this hierarchy. We propose that given the fundamental role of molecular processes to the structural and functional phenotype observed at the cell/tissue/organ/organism level, theoretically any change in apical end points will be preceded (i.e., driven) by a change at the molecular level. Epigenetic processes, such as expression of miRNAs, histone modifications, genomic imprinting, and DNA methylation, are fundamental to the expression of the transcriptome and proteome, and therefore we anticipate any apical effect observed following chemical exposure would be accompanied by an epigenetic change. Consequently, it is not surprising and is entirely consistent with current biological knowledge that an environmental chemical exposure at dose levels producing apical effects is associated with epigenetic changes [1,2].

We believe that with sufficient knowledge, epigenetic endpoints may be promising in at least two areas within a revised product safety assessment paradigm of the future. First, epigenomics could present a promising approach for BMD analysis to identify a dose level with no adverse toxicity potential (i.e. a POD), thereby potentially providing a revised approach for risk assessment. Second, changes in epigenetic endpoints could be used as surrogate biomarkers of traditional adverse apical effects or incorporated into adverse outcome pathways (AOPs) or MoA programs. The former would require a robust comparison of epigenomic and apical PODs, and the latter would require sufficient knowledge about how epigenetic changes are linked mechanistically to traditional apical end points. These two areas will be discussed in greater detail in sections 2 and 3.

2. Incorporating epigenetics into risk assessment

Marczylo et al. (2016) recently wrote: "The current body of evidence for environmentally induced epigenetic toxicity is predominantly a collection of human epidemiological data and exploratory *in vivo* high (often single) dose range studies, performed, not for regulatory purposes, but to investigate the theoretical potential and putative mechanisms of epigenetic toxicity in biological systems" [1]. While these investigative and mechanistic studies provide valuable data that contribute to the scientific field, the body of evidence lacks the necessary information to utilize epigenetic endpoints in an informed and consistent manner in product safety assessment. Namely, this is because with our current state of knowledge, it is difficult to link a specific epigenetic change to an adverse apical effect (i.e., establish causality) with sufficient scientific robustness for use in the regulatory decision making process.

The reported epigenetic transgenerational effects of vinclozolin (a fungicide PPP) is one example of a typical epigenetic study design in the toxicology literature, and serves as an interesting case study with regards to the risk assessment paradigm for protection of human health. Although there is conflicting evidence that exposure to vinclozolin elicits transgeneration epigenetic effects [3,4], the effects reported in the positive studies occurred at a dose level of 100 mg/kg/day (by intraperitoneal injection) [4–6]. For example, vinclozolin F3 generation rats (F0 dams were exposed to 100 mg/kg/day vinclozolin from embryonic day 8–14 of gestation) exhibited 52 promoter regions with statistically significant altered methylation patterns in the sperm compared to control samples [6]. Because only a single treated dose level was used, a transgenerational epigenetic effect POD was not identified. The 100 mg/kg/day dose level is orders of magnitude above the POD identified from the vinclozolin two-generation reproductive toxicity study [7,8]. While epigenetic endpoints were not included in the vinclozolin two-generation reproductive study, given the intersection of adverse apical outcomes and genomic and epigenomic changes,