published success rates for molecules entering phase 1 and phase 3 trials, and the proportion of drugs reported as having been assessed under ICH E14 Q5.1 and Q6.1 paradigms, we estimated that less than 10% of drugs entering clinical development will require a robust, sensitive *in vivo* QTc assessment. We have analyzed the resource requirements and performance of a sparse collection paradigm which could support entry into clinical testing. This initial assessment used sparse sampling from the full 24h data which was collected. The full 24h collected and stored data would allow complete and sensitive analysis for those molecules (<10%) entering phase 3 clinical testing and requiring more comprehensive nonclinical QTc information. **Methods:** The current project was focused on identifying the smallest amount of telemetry data required to maintain appropriate QTc analytical sensitivity for decision-making and ensuring human phase 1 clinical trial safety while reducing the overall time required for analysis in dogs and monkeys. Moxifloxacin was used as a reference agent for QTc effect detection. We re-examined the data from two previous studies (one study each in dogs and monkeys) which had included the reference drug and where complete data collection and analysis had occurred. We developed a sparser analysis paradigm for each species. In two additional studies (one in each species), the time taken to analyze the data, both via complete analysis and via the sparse analysis paradigm, was assessed. The primary measures for the study were the minimal detectable differences (analytical sensitivity) and the time taken for analysis (resource burden). **Results:** In both species the sparse sampling involved only 7 time points/animal/collection to represent the complete 24h collection. These included a pre-dose collection and then collections at time points to mimic plasma concentration sampling times. In monkey only 1 minute of data was used to represent the hourly time point. In dog there was a clear relationship between the amount of data collected in the hour and the minimal detectable difference. Five randomly chosen minutes within the hour were used to give a comparable performance (relative to complete analysis) when compared to monkey and full analysis. In both species the QTc prolonging effect of moxifloxacin was statistically significant, and the minimum detectable difference (n=4) was less than 10ms and 15ms in dog and monkey, respectively. In assessing analysis time, in both species 3 collections for each animal were evaluated. This would be match or exceed the number of collections for parallel QTc evaluations in standalone safety pharmacology studies and QTc data collection in toxicology studies. This assessment generates 3 files per animal per study. In monkey, each data file required on average only 4 minutes for assessment using the sparse analysis compared to 23 minutes for full analysis. The time taken for the sparse analysis was 82.6% less than for full analysis. In dog, the average time taken was 5 minutes with sparse analysis compared to 28 minutes for complete analysis. This represented an 82.1% reduction in analysis time. Across species, there was an ~82% saving in analysis time. **Conclusions:** When less than 10% of new molecules will require high sensitivity nonclinical QTc assessment, using the resource-sparing paradigm could reduce the time taken for QTc analysis prior to entry into clinical trials by 74% (0.9 x 0.82). This reduction in analyst time could significantly reduce the number of people, and the burden and cost of assessment of QTc analysis overall, while still including full data 24h collection. This supports the use of the highest quality data collection techniques (i.e., telemetry) while keeping analysis costs to the essential minimum.

PL 2005 Particulate Hexavalent Chromium Targets the Expression of Genes Involved in Lung Cancer and DNA Repair Pathways in Human Lung Cells

I. Meaza Isusi[1,2], *R. M. Speer*[1,2,3], *C. J. Kouokam*[1,2], and *J. P. Wise Sr.*[1,2]. [1]University of Louisville, Louisville, KY; [2]Wise Laboratory of Environmental and Genetic Toxicology, Louisville, KY; and [3]Department of Pharmacology, University of New Mexico, Albuquerque, NM.

Background and Purpose: Hexavalent chromium [Cr(VI)] is a well-established human lung carcinogen with widespread environmental and occupational exposures. Cr(VI) causes DNA double-strand breaks and loss of DNA break repair, leading to chromosome instability, which is a driving mechanism in Cr(VI) carcinogenesis. However, the ability of particulate Cr(VI) to inhibit DNA break repair across repair pathways is poorly understood. Our previous data show Cr(VI) alters the expression of mRNA related to DNA double-strand break repair; however, these studies focused on individual genes and did not assess the broader impact of Cr(VI) on repair genes across the genome. Thus, the aim of this study was to characterize global transcriptional changes in mRNA expression and specifically consider lung cancer and DNA repair pathways in human lung cells after acute (24 h) and prolonged (>72 h) particulate Cr(VI) exposures. **Methods:** Normal human lung fibroblast cells were exposed to several concentrations (0.1, 0.2 and 0.3 ug/cm^2) of zinc chromate, a representative particulate chromate compound known to cause lung cancer in humans. Cells were exposed to acute (24 h) or prolonged (72 h or 120 h) zinc chromate exposures. At the end of treatment, cells were harvested, and RNA was extracted, followed by RNA sequencing. Clarivate's MetaCore bioinformatics software suite was used for molecular and disease pathway analyses. Gene expression heatmaps were developed using Partek's bioinformatics software. Cr(VI)-altered mRNAs and miRNAs were analyzed using miRSystem database. The RNA and miRNA datasets were validated using qPCR. **Results:** Our results show exposure to particulate Cr(VI) induced a time- and concentration-dependent increase in the total number of upregulated and downregulated genes. Using Clarivate's MetaCore bioinformatics software suite, we compared our list of Cr(VI)-targeted genes with disease databases and found Cr(VI) altered the expression of genes involved in lung cancer and lung-associated diseases, consistent with its known ability to induce lung cancer and lung disease. We also found Cr(VI) altered the expression of genes involved in key DNA repair and DNA maintenance pathways. To further investigate the effect of Cr(VI) on specific genes involved in DNA repair pathways, we developed a list of genes involved in each pathway based on KEGG, GO and literature sources. Based on these gene lists gene expression heatmaps of 8 different DNA repair pathways were generated: 1) homologous recombination repair, 2) non-homologous end joining, 3) microhomology-directed end-joining, 4) single strand annealing, 5) mismatch repair, 6) base excision repair, 7) nucleotide excision repair and 8) DNA crosslink repair. Our data show Cr(VI) induced global downregulation of genes involved in all eight DNA repair pathways. We also considered miRNAs, short length RNAs that can modulate gene expression, as a possible regulatory mechanism for Cr(VI)-induced transcriptomic changes. We compared our miRNA and mRNA datasets, and Cr(VI)-altered miRNAs were predicted to target Cr(VI)-modulated mRNAs, suggesting Cr(VI) may target miRNAs to alter gene expression of key genes. Specifically, we used the miRSystem database to identify miRNAs predicted to target key genes in DNA repair pathways such as, RAD51, RAD51B, MLH3, and XRCC5. We found the expression of a number of the miRNAs predicted to target these genes were altered by particulate Cr(VI) exposure. **Conclusions:** Altogether, our data show Cr(VI) exposure in human lung cells leads to differential expression of genes involved in lung cancer and induces a global downregulation of genes involved in high-fidelity DNA repair pathways, which can lead to chromosome instability and cancer. Additionally, we also show miRNA modulation as a potential regulatory mechanism of Cr(VI)-induced transcriptomic changes. This work was supported by NIEHS grants R01ES016893 (JPW), R35ES032876 (JPW), and T32ES011564 (RMS and JPW).

PL 2006 Systematic review of the mechanistic evidence for TiO_2 NP lung carcinogenicity

S. Wolf[1], *K. Sriram*[2], L. M. Camassa[1], D. Pathak[2], H. L. Bing[1], B. Mohr[1], S. Zienolddiny-Narui[1], and J. Samulin-Erdem[1]. [1]National Institute of Occupational Health, Oslo, Norway; and [2]NIOSH, Morgantown, WV.

Background and Purpose: Titanium dioxide nanoparticles (TiO_2 NP) are a high production volume material widely used in the paints, cosmetic, food and photovoltaics industry. Their increasing industrial demand and use raises concerns of adverse human exposure and related health effects. Epidemiological and animal studies suggest that TiO_2 elicits cancer development and consequently is classified as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer. However, the mechanistic basis of TiO_2 NP-mediated lung carcinogenicity is not clear. Here, we systematically reviewed literature to identify *in vitro* and *in vivo* mechanistic evidence of TiO_2 NP lung carcinogenicity using the 10 key characteristics (KC) of carcinogens for identifying and classifying carcinogens. **Methods:** Based on the 'Population, Exposure, Comparator, Outcome and Study' framework, a systematic literature search of studies published between 2006 and 2023 was performed using the four databases PubMed, Embase, Web of Science and TOXicology information onLINE to identify existing *in vitro* and *in vivo* data on the mechanisms of TiO_2 NP toxicity relevant to the onset of lung carcinogenicity. The 10 KC of carcinogens were comprised in the following six search strings: 'Genotoxicity', 'Oxidative stress', 'Chronic inflammation', 'Epigenetic effects', 'Proliferation, Apoptosis' and 'Receptor-mediated effects'. Two independent reviewers did the title/abstract and full-text screening by applying pre-defined inclusion and exclusion criteria. The quality and reliability of the studies was assessed by using the ToxRTool, which resulted in the assignment of modified Klimisch scores. Studies receiving a Klimisch score 1 or 2 were eligible for data extraction which followed standardized forms and guidelines. Finally, a weight-of-evidence approach was used to evaluate the assays/endpoints investigated to study the respective KC in terms of their association with carcinogenic hazard. **Results:** The systematic literature search yielded a total of 15,489 articles, which were screened for title/abstract and 1,842 articles were assessed for eligibility in the full-text screening. After curating the reference database (i.e., removal of overlapping studies in the six search strings), 346 articles were included in the quality and reliability assessment using the ToxRTool, of which 262 articles reported *in vitro* data and 102 articles reported *in vivo* data. Of these, 186 articles were considered of good quality and provided moderate to high level of confidence for the biological endpoints examined. Our analysis showed limited availability of mechanistic data related to carcinogenesis, such as proliferation, epigenetic alterations and receptor-mediated effects. There was evidence for genotoxicity, oxidative stress and chronic inflammation following TiO_2 NP exposure. However, the wide variety of TiO_2 NP used in the studies, differing in their physicochemical characteristics, methods of formulation, exposure scenarios/test systems, and experimental protocols made it very challenging to compare the studies. **Conclusions:** Future toxicology/carcinogenicity research must include appropriate positive controls, endotoxin testing, statistical power analysis, and relevant biological endpoints, to improve the quality and reliability of the studies to be useful for the evaluation of TiO_2 NP-induced lung carcinogenicity. There is clearly a need for more physiologically

relevant, long-term studies using appropriate particle doses. The limited quality of currently available evidence constrains us from drawing a conclusion that pulmonary exposure to TiO_2 NP induces lung cancer.

2007 Deciphering the antagonistic effects of AhR and NRF2 on lung carcinogenicity of 1,4-dioxane

Z. Wang, Z. Bi, Y. Qiu, A. Pawar, W. Zhang, H. Ji, C. Thakur, and F. Chen. Stony Brook Cancer Center, Stony Brook, NY.

Background and Purpose: 1,4-dioxane (1,4-D) has been considered as a probable human carcinogen, and its contamination in drinking water is an emerging public health concern in the US. Currently, limited animal studies indicate that 1,4-dioxane facilitated tumor initiation. However, the evidence of direct mutagenicity and carcinogenicity of 1,4-D is still insufficient in humans, and it is urgently needed to clarify the involving mechanisms in this process. **Methods:** In this study, we developed a cellular model by treating human bronchial epithelial cells (BEAS-2B) with 1.25–20 ppm 1,4-D for 3 months. The assessment of dose-dependent carcinogenicity of 1,4-D was conducted using colony formation assay and tumorigenicity assay in nude mice. Bulk RNA sequencing (RNA-seq), chromatin immunoprecipitation and sequencing (ChIP-seq), CRISPR-Cas9 gene editing, and functional analysis were performed to identify the associated mechanism involved in the carcinogenicity of 1,4-D, with a specific focus on the mutual antagonistic effects of aryl hydrocarbon receptor (AhR) and NRF2. **Results:** Our results indicated the carcinogenicity of 1,4-D at environmentally relevant concentrations. Immunoblotting revealed that 1,4-D was potent in activating the oncogenic transcription factor NRF2 within a dose range of 2.5 to 20 ppm. Conversely, exposure to 1,4-D decreased the protein level of AHR within the same dose range. ChIP-seq analysis showed that AHR was normally enriched on several oncogenic genes in addition to the known phase I/II enzymes, including those in TGFβ and Nrf2 signaling pathways, as well as various known oncogenes. Upon 1,4-D treatment, the binding of AHR to these genes was substantially reduced, leading to an increased expression of these genes. In parallel, we conducted cell transform assay on *NRF2* knockout (*NRF2* KO) and *AhR* knockout (*AhR* KO) cells to investigate the interaction between AhR and NRF2. Our data suggested that *NRF2* knockout reduced the carcinogenic latency of 1,4-D, while *AhR* knockout increased it, in comparison to the point of departure (PoD) of 1,4-D carcinogenicity in wild-type (WT) cells. Furthermore, gene profiling of the WT, *NRF2* KO and *AhR* KO cells via RNA-seq suggested a pronounced induction of genes in the pathways of protein degradation, oncogenesis, and epithelial-mesenchymal transition (EMT) in a NRF2-dependent manner. *AhR* knockout, on the other hand, enforced the expression of the NRF2-dependent 1,4-D-induced genes, indicating an antagonistic role of AhR in Nrf2 signaling. **Conclusions:** Taken together, the findings from this report provide the first evidence indicating that 1,4-D is a human carcinogen, and its carcinogenicity is strongly reliant on 1,4-D-mediated NRF2 activation. Significantly, this study fills the existing knowledge gaps in our comprehension of the carcinogenic potential of 1,4-D and offers fundamental data to policymakers for the assessment and regulation of environmental contamination by 1,4-D.

2008 Estrogenic endocrine disrupting compounds increase cell viability and cancer stem cell formation in estrogen receptor positive breast cancer cell lines

C. Winz, K. C. Lee, S. Rohatagi, E. Li, P. Furmanski, and N. Suh. Rutgers University, New Brunswick, NJ.

Background and Purpose: Estrogenic endocrine disrupting compounds (EDCs) found in plastics, drinking water, and food, pose a threat to human health. Estrogenic EDCs share chemical similarity with the endogenous hormone estrogen, and are thus able to activate estrogen receptor (ER) related signaling pathways. Excessive estrogen signaling is a key hallmark of ER+ breast cancer. In this breast cancer subtype, estrogen signaling can induce proliferation, migration, and the formation of breast cancer stem cells (CSCs). CSCs are a small subpopulation of cancer cells that are uniquely capable of repopulating tumors *in vivo*. Clinically, increased numbers of CSCs have been associated with metastasis and recurrence in breast cancer patients. Since estrogenic EDCs share chemical similarity with E2, they may also induce cancer stemness in ER+ breast cancer, resulting in poorer disease outcomes. We hypothesized that estrogenic EDCs increase cell proliferation of ER+ breast cancer cell lines and induce cancer stem cell formation. **Methods:** Two estrogenic endocrine disruptors routinely found in human biosamples were selected for analysis: bisphenol-A (BPA) and alpha-zeranol (aZAL). MTT assays and qPCR were employed to assess the estrogenic and growth promoting effects of these EDCs in a panel of ER+ and ER- breast cancer cell lines. To assess cancer stemness, a unique *in vitro* mammosphere formation model was utilized. Breast cancer cell lines were seeded in ultra-low attachmant plates to promote only the survival of CSCs. Resulting CSCs form spheres of repopulated cells that were collected and assessed. Size and quantity measurements were taken and flow cytometry was performed to assess the expression of stem cell markers. **Results:** In the ER+ MCF7 cell line, E2, BPA, and aZAL significantly increased cell viability by 71.5 +/- 7.17%, 61.5% +/- 3.8%, and 73.6% +/- 16.9% respectively at the 72-hour timepoint. In the ER+ cell line T47D, similar significant increases in cell viability were seen. The ER- cell lines DCIS and MDA-MD-231 exhibited no changes in viability after EDC treatment, indicating the estrogen receptor is likely needed for growth promotion by EDCs. In addition, we found that the expression of the estrogen target genes PGR and TFF1 were significantly induced following treatment with BPA or aZAL in both ER+ cell lines, but neither of the ER- cell lines. To enumerate stem cell formation following treatment, MCF-7 cells were treated with either E2 or EDC and subsequently placed in mammosphere conditions. Both BPA and aZAL significantly increased the size and quantity of resulting mammospheres. Additionally, BPA and aZAL increased the proportion of cancer stem cells, defined as the $CD24^{low}$ $CD44^{high}$ expressing subpopulation. **Conclusions:** Overall, our results indicate that the estrogenic EDCs BPA and aZAL increase ER+ breast cancer proliferation, stem cell formation, and sphere recolonization. This is especially concerning for breast cancer patients that have environmental or occupational exposures to these EDCs. There is limited epidemiological data on how EDCs affect disease prognosis of breast cancer patients. More research is needed to further elucidate the molecular mechanisms that could drive EDC-induced cancer promotion. Our research suggests that estrogen-signaling induced cancer stemness may be a mechanism of interest.

2009 Chemicals that induce mammary tumors in rodents have an increased probability of stimulating estradiol and progesterone synthesis, activating the ER, and causing genotoxicity *in vitro*

J. E. Kay[1], J. G. Brody[1], M. Schwarzman[2], and *R. A. Rudel*[1]. [1]Silent Spring Institute, Newton, MA; and [2]University of California, Berkeley, School of Public Health, Berkeley, CA.

Background and Purpose: Hundreds of chemicals are plausibly linked to breast cancer (BC) based on evidence that they increase mammary tumors in animals or increase estrogen or progesterone signaling, activating hormonal pathways known to influence risk. However, relatively few of the 42,000+ chemicals used in commerce in the US have been tested in a rodent cancer bioassay, the current standard for carcinogen classification. To protect humans from harmful exposures, efficient and modernized approaches are needed to identify potential breast carcinogens and prioritize the most hazardous chemicals for exposure reduction. Here, we applied a Key Characteristics approach to identify chemicals with mechanistic activities associated with increased BC risk. Based on strong evidence that mutagenic DNA damage and increased estrogenic and progestogenic signaling increase BC risk, we aimed to systematically identify chemicals with these activities from databases for genotoxicity and high throughput screens for activity at the estrogen receptor (ER) and increasing hormone biosynthesis. We also compiled rodent mammary carcinogens identified in cancer bioassays and evaluated how well data on ER activity, steroidogenesis, and genotoxicity could predict whether a chemical is likely to induce mammary tumors and, by inference, increase BC risk. **Methods:** We identified ER agonists and their magnitudes of effect published by Judson et al. (*Tox Sci*, 2015) using computational integration of 11 ER-related assays in US EPA's ToxCast screening program. We identified chemicals that stimulated estradiol (E2) and/or progesterone (P4) synthesis in ToxCast H295R screening and, when possible, their magnitudes of effect as published by Karmaus et al. (*Tox Sci*, 2016), Haggard et al. (*Tox Sci*, 2018), and Cardona and Rudel (*EHP*, 2021). We identified rodent mammary carcinogens from carcinogenicity testing as reported by the EPA, NTP, IARC, Lhasa Carcinogenicity Database, and our previous publication Rudel et al. (*Cancer*, 2007). We then used EPA, NTP, OECD, and EU databases to classify the genotoxicity of each of the 921 endocrine disrupting and/or mammary carcinogenic chemicals. Finally, we compared the enrichment of endocrine-disrupting and genotoxic activities of rodent mammary carcinogens to other tested chemicals to determine how well this mechanistic data can predict *in vivo* effects. **Results:** Together, we identified 921 agents that either induced mammary tumors in rodents (279), increased E2 or P4 biosynthesis (515), and/or activated the ER (267), and 421 of these were also genotoxic. To test the utility of ER activity, steroidogenesis, and genotoxicity to predict chemicals' ability to increase BC risk, we compared these mechanistic activities among rodent mammary carcinogens to those of 1) all chemicals tested in those assays, and 2) chemicals that did not induce mammary tumors in the standard 2-year cancer bioassay. Consistent with our hypothesis, rodent mammary carcinogens were significantly more likely to be ER agonistic, E2 or P4 steroidogenic, genotoxic, and have combinations of these activities relative to both comparison groups. We observed a dose-response trend: mammary carcinogens were 2.6-fold more likely to be strong endocrine disruptors compared to non-mammary carcinogens, vs. 1.6-fold for moderate endocrine activity. Endocrine disruption combined with genotoxicity was even more strongly enriched among mammary carcinogens (3-fold). However, many mammary carcinogens were not active in these endocrine assays or had not been tested. These two endocrine assays had high specificity, with most non-mammary carcinogens testing negative, but low sensitivity, with many mammary carcinogens testing negative as well. **Conclusions:** These findings support the use of mechanistic evidence to identify potential breast carcinogens. Mammary carcinogens were enriched for ER agonistic, steroidogenic, and genotoxic activities, consistent with the Key Characteristics framework and known BC risk factors. Our

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