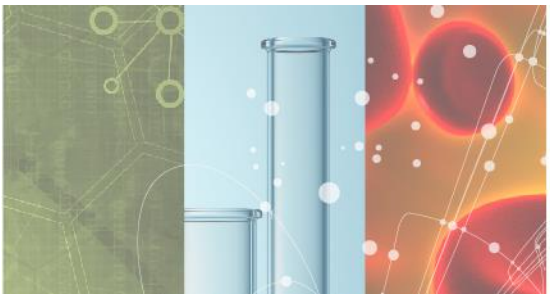

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American Chemistry Council

ACC Science and Research Highlights

Research on Understanding Mode of Action to Improve the Scientific Basis of Risk Assessment

Introduction and Background

In the 1970's, when knowledge was lacking on how chemicals act in the body to produce toxicity, the first quantitative methods to estimate human cancer risk typically extrapolated from observational data in high dose lab animal studies using conservative, default assumptions. It was presumed at that time that any degree of exposure, no matter how low, posed some level of risk. In other words, risks could be extrapolated from the responses observed in high dose animal studies by extending the dose response line down through zero. (This method is still in use today in some programs, even though research over the past four decades has greatly increased understanding of modes of action— knowledge of how chemicals act at the molecular/cellular/organ levels to cause toxicity.

Advancements Made in Scientific Knowledge of Chemical Effects on Biological Systems: Use of Mode of Action in Evaluating Carcinogenic Risks

As toxicology transitioned to a mechanistic discipline, it became increasingly apparent that the process of carcinogenesis involves multiple steps and many biological pathways, that different types of chemicals operate via different pathways, that not all tumors in animal studies are relevant to humans and the method for extrapolating risks to humans should be based on an understanding of modes of action.

Mode of action (MOA) information describes the key events comprising the sequential steps and processes involved in toxicity. There are many examples of MOAs for carcinogenesis, such as mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression. (Note – MOA is distinguished from "mechanism of action," which implies a more detailed understanding and description of each and every event, often at the molecular level.)

Promoting the thoughtful application of MOA information to improve the scientific basis of chemical risk assessments is a core activity of ACC. The development and evolution of the WHO/IPCS MOA Framework has helped pave the way for implementing a systematic approach for integrating evidence from mechanistic studies, animal experiments, and human epidemiology investigations. However, movement away from default assumptions has progressed unevenly in different regulatory programs. In some programs MOA evaluations are fairly routine. But in others, at times it seems that overcoming the conservative defaults requires both absolute proof of a MOA and disproving of default assumptions – an impossible task for science.

ACC's Commitment to Improving Scientific Knowledge of Mode of Action to Enhance the Accuracy of Human Health Risk Assessments

In addition to the efforts of ACC's Center for Advancing Risk Assessment Science and Policy (ARASP) in catalyzing incorporation of the best available scientific methods for integrating evidence and incorporating MOA analyses into to risk assessments, many chemical-specific panels in ACC's Chemical Products and Technology Division have devoted significant resources to investigate and evaluate MOAs of their chemicals with the goal of providing scientifically solid data and analyses to improve the accuracy of risk assessments. The activities of three ACC panels are highlighted below.

ACC Science and Research Highlights is a publication for ACC members and staff to communicate information on scientific research projects conducted or sponsored by ACC departments

by Richard A. Becker Ph.D., DABT, ACC's Science and Research Division, Regulatory & Technical Affairs Department

Formaldehyde Panel

Role of Endogenous Exposures in Human Health Assessments

An understanding of the effects of background levels or endogenous concentrations is an important element in characterizing the shape of the dose-response curve in the low-dose region. However, conducting a chemical assessment for a substance that is present endogenously can pose several challenges. First, methods are needed to quantify endogenous production and impacts from biochemically identical damage arising from exogenous exposure. Once such methods are developed and results obtained, an additional challenge is determining how to best interpret the results and incorporate those results into an appropriate dose-response assessment.

Key Differences Found in Endogenous and Exogenous Formaldehyde Exposures

Background: Formaldehyde is present endogenously in all living cells and it is an essential metabolic intermediate. It also has several exogenous sources including vehicle emissions, off-gassing from building materials, tobacco smoke; and is produced from the metabolism of foods. In order to improve understanding of the mode of action of potential human health risk from formaldehyde exposures, it is important to quantify the contribution of formaldehyde produced by the body. This research examines and evaluates the formation, accumulation, and hydrolysis of exogenous and endogenous formaldehyde exposures

Study Findings: In this study, a biomarker (e.g. N2-HOMe-dG) was utilized to evaluate direct adduction of formaldehyde to DNA and the hydrolysis of DNA protein crosslinks. The use of inhaled [13CD2]-formaldehyde exposures of rats and primates coupled with ultrasensitive liquid chromatography-tandem mass spectrometry permitted accurate determinations of endogenous and exogenous formaldehyde DNA damage. The results show that inhaled formaldehyde only reached rat and monkey noses, but not tissues distant to the site of initial contact. The amounts of exogenous adducts were remarkably lower than those of endogenous adducts in exposed nasal epithelium. Moreover, exogenous adducts accumulated in rat nasal epithelium over the 28-day exposure to reach steady-state concentrations, followed by elimination with a half-life (t1/2) of 7.1 days. Below is one table included in the publication that illustrates the notable differences in the rat.

TABLE 1. Formation of N^2-HOMe-dG Mono-Adducts (mean ± SD) in Rat Nasal Epithelium, Bone Marrow, and White Blood Cells Exposed to 2 ppm Labeled Formaldehyde for 28 Days

Exposure Period	Rat Nasal Epithelium			Rat Bone Marrow			Rat White Blood Cells		
	N^2-HOMe-dG (adducts/10^7 dG)			N^2-HOMe-dG (adducts/10^7 dG)			N^2-HOMe-dG (adducts/10^7 dG)		
	Endogenous[a]	Exogenous	n	Endogenous[a]	Exogenous	n	Endogenous[a]	Exogenous	n
7 days	2.51 ± 0.63	0.35 ± 0.17	5	3.37 ± 1.56	n.d.	6	2.62 ± 1.12	n.d.	4
14 days	3.09 ± 0.98	0.84 ± 0.17	5	2.72 ± 1.36	n.d.	6	2.26 ± 0.46	n.d.	4
21 days	3.34 ± 1.06	0.95 ± 0.11	5	2.44 ± 0.96	n.d.	6	2.40 ± 0.47	n.d.	4
28 days	2.82 ± 0.76	1.05 ± 0.16	6	3.43 ± 2.20	0.34[b]	12	2.49 ± 0.50	n.d.	4
28 days + 6 h postexpo	2.80 ± 0.58	0.83 ± 0.33	9	2.41 ± 1.14	n.d.	6	2.97 ± 0.58	n.d.	4
28 days + 24 h postexpo	2.98 ± 0.70	0.80 ± 0.46	9	4.67 ± 1.84	n.d.	5	2.57 ± 0.58	n.d.	4
28 days + 72 h postexpo	2.99 ± 0.63	0.63 ± 0.12	9	5.55 ± 0.76	n.d.	6	1.75 ± 0.26	n.d.	4
28 days + 168 h postexpo	2.78 ± 0.48	0.67 ± 0.20	10	2.78 ± 1.94	n.d.	4	2.61 ± 1.22	n.d.	4
Air control	2.84 ± 0.54	n.d.	8	3.58 ± 0.99	n.d.	6	2.76 ± 0.66	n.d.	6

[a]No statistically significant difference was found using the 2-sided Dunnett's test (multiple comparisons with a control) (Dunnett, 1964).
[b]The amount of exogenous N^2-HOMe-dG adducts that was found in only 1 bone marrow sample analyzed by AB SCIEX Triple Quad 6500.
n.d., not detected.

Implications for Chemical Assessment: These findings provide critical new data for understanding the role of endogenous exposure of formaldehyde. Results from the study improve our ability to develop science-based chemical assessments utilizing known biology and toxicology information, rather than relying on default approaches. The results suggest that chemical risk may be overestimated for inhaled formaldehyde and indicates that endogenous formaldehyde may be a primary source of exposure.

Citation: Yu, Rui, Yongquan Lai, Hadley J. Hartwell, Benjamin C. Moeller, Melanie Doyle-Eisele, Dean Kracko, Wanda M. Bodnar, Thomas B. Starr, and James A. Swenberg. "Formation, accumulation, and hydrolysis of endogenous and exogenous formaldehyde-induced DNA damage." Toxicological Sciences 146, no. 1 (2015): 170-182.

This work was supported in part through the Foundation for Chemistry Research and Initiatives. For more information about this important science research and how you can engage on formaldehyde related science, please email: Kimberly_White@americanchemistry.com.

Hexavalent Chromium Panel

Mode of Action Research on Hexavalent Chromium Suggests Non-Mutagenic MOA

Classic 2-year bioassays, generally performed with high doses in rodents, provide key insights into potential hazards posed by chemicals but, by design, do not provide significant information regarding the MOA by which a chemical caused a carcinogenic response. The MOA is an important component to consider when evaluating the cancer risk posed by a chemical. Hexavalent chromium (Cr6) is present at low ppb levels in groundwater, the bulk of which originates from geologic processes. A NTP drinking water 2-year bioassay for Cr6 reported small intestine (SI) tumors in mice (but not rats) at the highest doses administered. These findings triggered questions about how the SI tumors formed in mice (i.e., what was the MOA) including whether these high dose effects could occur at environmental levels of less than 100 ppb (the federal total chromium drinking water standard), and whether these high dose animal studies have biological relevance to humans. To answer these questions, a set of MOA studies were conducted with mice and rats of the same strains tested in the NTP study. Animals were administered Cr6 at the four concentrations tested by NTP, and at two lower concentrations (equivalent to 100 and 1,400 ppb), which are more relevant to human exposures. Data were collected to identify how these tumors occurred and to build PBPK models to extrapolate high dose rodent data to low doses and to inform risk (if any) to humans at low doses. The Cr6 MOA research studies were developed to provide data to evaluate which proposed MOA best fit the available data – a mutagenic MOA proposed by McCarroll *et. al.* (2010),[1] or a non-mutagenic MOA proposed by Thompson *et al.* (2011),[2] which was developed in accordance with the MOA framework outlined in EPA's 2005 Guidelines for Carcinogenic Risk Assessment. The MOA research included studies investigating toxicogenomics, genotoxicity, and pharmacokinetics.

With over 20 manuscripts published in the last 5 years, the MOA research has added new data to that from the NTP study to provide a robust public database, including target species and tissue specific data, for science policy and regulatory decision-makers.

Table 1. MOA Study Findings (Mice)

Significant Change	Cr6 Drinking Water Concentration (mg/L)					
Day 91 Duodenum	0.1	1.4	5	20	60	180
Cr in Duodenum	No Effect in the Low Dose Range		✓	✓	✓	✓
Oxidative Changes			✓	✓	✓	✓
Gene Changes			✓	✓	✓	✓
Villus toxicity			-	✓	✓	✓
Crypt proliferation			-	✓	✓	✓
Crypt DNA damage	No Mutagenesis and No Basis for Linear Low Dose Extrapolation					
***K-Ras* mutation (Codon 12 GAT)**						

Underlined checks indicate significant changes at day 8 as well, Cr concentrations not measured at day 8.

Effects are consistent with a chronic wound healing mode of action.

The target tissue specific data indicate that gastric fluid efficiently reduces Cr6 to non-toxic Cr3 and support a multiple pool reduction model. Importantly, the research shows that environment levels of Cr6 in drinking water are readily reduced in the stomach. The research suggests that the high experimental doses exceed the reductive capacity and rate in the stomach and spill over to the SI. Investigations on the SI tumors in mice using *in vivo* and innovative imaging studies indicate that there is no genotoxicity in the crypt compartment suggesting that the stem cells are not impacted by Cr6. The genotoxicity data and a pathology reanalysis of the stored NTP and MOA tissues suggests that proliferation in the crypt is an early signal for villus cell loss resulting in blunting. These data are similar to captan and folpet, which EPA's Office of Pesticide and Prevention classifies as non-mutagenic.

Collectively, the NTP and MOA research provide strong evidence that Cr6 appears to have a threshold and the SI tumors originated as a result of a non-mutagenic MOA. This has important implications for how a risk assessment is conducted for Cr6.

See http://cr6study.info/ for more information about the Cr6 studies, access to the publications and access to the research data/findings.

[1] McCarroll, et al., 2010. An Evaluation of the Mode of Action Framework for Mutagenic Carcinogens Case Study II: Chromium (VI). Environmental and Molecular Mutagenesis. Available here.

[2] Thompson, et al., 2011. Application of the U.S. EPA Mode of Action Framework for Purposes of Guiding Future Research: A Case Study Involving the Oral Carcinogenicity of Hexavalent Chromium. *Toxicological Sciences*. 119(1): 20-40. Available here.

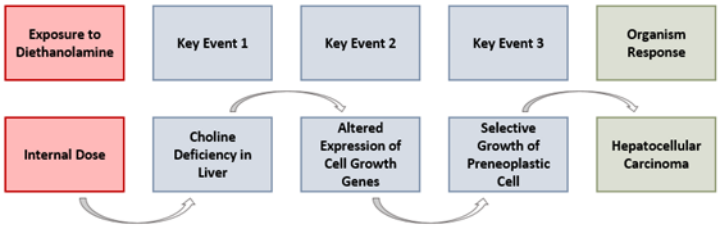
Most of the MOA research was supported with funding from the Hexavalent Chromium panel of the American Chemistry Council. MOA researchers also received funding for some studies from other sources including the Electric Power Research Institute (EPRI). For more information about this research and how you can engage on Cr6 related science, please email: ann_mason@americanchemistry.com.

Alkanolamines Panel

Background

Diethanolamine (DEA) is used as an intermediate in the production of herbicides and personal care products and in natural gas treatment applications. Dermal exposure to DEA has been shown to increase liver tumors in B6C3F1 mice. DEA is not DNA reactive (non-genotoxic) in both *in vitro* (with and without metabolic activation) and *in vivo* assays. In our analysis of DEA-induced mouse liver tumors, we concluded that the data do not support a mutagenic MOA for DEA liver carcinogenicity. We also evaluated the hypothesis that DEA induces mouse liver tumors via choline deficiency, a non-mutagenic MOA. Choline is an essential nutrient for maintaining methylation status, phospholipid biosynthesis, and cell signaling. The postulated key events (KE), supported by published data, are depicted in Figure 1.

Figure 1. Key Events: Choline Deficiency MOA



Carcinogenicity Evaluations of DEA

- 2-year NTP dermal exposure study in B6C3F$_1$ mice: clear evidence of carcinogenicity in male and female mouse livers and male kidneys. Human relevancy of these findings is not known.
- IARC 1999: concluded DEA was not classifiable as to its carcinogenicity in humans.
- IARC re-evaluation 2012: sufficient evidence in experimental animals; overall evaluation of possibly carcinogenic to humans (Group 2B).

Table 1. Summary of Potential MOAs

MOA	Weight of Evidence in Rodents	Relevance to Humans (If Occurs in Rodents)
Genotoxicity	Not supported	Relevant
Choline-deficiency	Data generally supportive, but there are a number of inconsistencies and data gaps	Not relevant due to qualitative differences
Cytotoxicity	Not supported	Relevant
Inflammation	Not supported	Relevant
Infection	Not supported	Relevant
Oxidative stress	May be a modulating factor or associative event	Relevant
Receptor-mediated[1]	Limited evidence against	May be relevant (depending on receptor and dose)

Table 2. Key Events: Evidence Related to Choline Deficiency

Key Event (KE)	Data from In Vitro/In Vivo Studies	Human Relevancy Considerations
Internal Dose	Greater percutaneous absorption through skin of laboratory animals based on percent absorbed in 6 hours (Sun et al. 1996). Human (0.23%) < Rat (0.56%) < Mouse (6.68%)	Based on animal studies, tissue distribution, regardless of route of exposure, highest in liver and kidney (Matthews et al 1997). Competes with choline in phospholipid biosynthesis
KE1: Choline Depletion	S-adenosyl methionine (SAM) and choline depleted at tumorigenic doses	Choline oxidase levels high in rat and mouse, low in humans. May account for species sensitivity and dependence on exogenous choline (Sidransky and Farber 1960). Forms methyl-DEA potentially further depleting SAM and subsequent methylation reactions
KE2: Altered Expression of Cell Growth Genes	Changes in cell growth genes and DNA synthesis in vivo at tumorigenic doses (Kamendulis and Klaunig 2005).	Alternate mechanisms exist in humans to supply methyl groups and sustain methylation reactions.
KE3: Selective Growth of Preneoplastic Hepatocytes	Inferred from increased DNA synthesis *in vivo* and high incidence of initiated hepatocytes in B6C3F$_1$ mice in NTP study. Hepatocellular proliferation in mice (Mellert 2004).	No data. However, since SAM levels are largely unaffected, perturbations in methylation status is unlikely to drive a proliferative response.
Liver Tumors	Positive results in B6C3F$_1$ mouse bioassay	Lack of epidemiologic data associating liver cancer and choline deficiency in human populations

Conclusions

1. A genotoxic MOA is not supported based on the current evidence.
2. The most supported MOA for DEA is choline deficiency although certain data gaps exist. According to this MOA, DEA has threshold response and various species differences indicate tumorigenicity in rodents has limited relevance to humans
3. Species differences include rates and degree of percutaneous absorption and various cellular compensatory mechanisms whereby humans are more refractory to choline deprivation.

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