

2023 Annual Report

Director:

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ABOUT CRIS

The Center for Research on Ingredient Safety at Michigan State University (CRIS) is one of the few organizations in the world willing to tackle the hard questions about ingredient safety in our everyday products.

We are unique in that we work with academia, government, nongovernmental organizations, and industry to understand the safe use of ingredients. To be a CRIS Partner, an entity must be willing to submit to our code of ethics of complete transparency and always put the consumers' best interests regarding ingredient safety first.

But how do we ensure these entities have our best interests at heart? How do we know that what they are saying about the ingredients in our everyday products is true?

CRIS fills that void. We use expert knowledge to research, factcheck, and supply the global community with the latest sciencebased information about the ingredients in our food, beverages, and cosmetics.

When data gaps exist, CRIS conducts laboratory-based research to obtain information on ingredient safety.

We leverage the expertise of established investigators who have devoted their lives work to science, and we demystify dense academic journal findings and governmental reports so you can make informed decisions about the science in our lives.

While this may make us unpopular with some groups, we believe that transparency and truth are requirements to earn your trust, and the confidence of the global community.



MISSION & VISION

Our <u>mission</u> is to conduct research and provide insight into the safety of ingredients in food and consumer products to support evidence-informed decisions by consumers, industry, and policymakers.

Our <u>vision</u> is to ensure credible, relevant information on ingredient safety is accessible to a wide range of decision-makers.

CORE VALUES

We operate using our core values:

- Integrity
- Responsibility
- Transparency
- Inclusivity
- Engagement
- Diversity

THE OBJECTIVE

We at the Center for Research on Ingredient Safety at Michigan State University (CRIS) strive to become the go-to source for information on ingredient safety.

OUR COMMITMENT & OUR ETHICS

We at the Center for Research on Ingredient Safety at Michigan State University (CRIS) believe that science and research should speak for itself. That is why we wrote transparency and accountability into our bylaws and governance practices from CRIS's inception to ensure that we

- produce and disseminate unbiased and credible research data and analysis on the safe use of ingredients in food and consumer products.
- offer unbiased and transparent evaluation of new technology that can be applied to evaluate ingredient safety.
- expand the opportunity to conduct basic and applied research on the safety and toxicology of ingredients in food and consumer products to support the management of potential safety issues.

This also means that the established investigators conducting research at CRIS have final say on all scientific research, including

- driving the research agenda.
- research design, methods, and conduct.
- interpreting and publishing findings in peer-reviewed journals.

To ensure impartiality, all research outcomes from CRIS-conducted projects must undergo the peer review process and are published. Research findings are not shared, including with members, or communicated until accepted for publication in the peer-reviewed literature. All of our research methods and processes are available openly and freely for any person to evaluate.

While our partners can participate in CRIS advisory committees, all final research and communication decisions are made by the CRIS team using the CRIS bylaws and established governance.

Additionally, we follow the code of ethics outlined by Michigan State University.

OUR MEMBERS





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COMPANY







OUR TEAM

The Center for Research on Ingredient Safety at Michigan State University (CRIS) consists of a core team of individuals who provide leadership, management, research, educational opportunities, and direction for CRIS activities. In addition to our core team, we're connected to a global network of scientists and researchers available to support CRIS research.

These activities are guided by input from CRIS advisory committees. However, all final decisions are made by the CRIS team.



Norbert Kaminski, Ph.D.

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A NOTE FROM THE DIRECTOR

As we reflect on the year 2023, I am proud to share the significant achievements and strides we have made at CRIS. This year has been marked by several key milestones that not only underscore our commitment to advancing our mission at CRIS but also enhance our collective capabilities to tackle new and emerging challenges.

One of our major triumphs this year includes securing an Alliance Grant with Corewell Health. This funding bolstered our research capabilities, providing us with the resources needed to embark on a project to use our developmental immunotoxicology (DIT) assay. This assay uses stems cells from human umbilical cord blood, which for this study will come from participants in our Michigan community to evaluate how lead impacts the developing immune system. Although there is significant interest and concern in heavy metals and their effects on human health, an added benefit of this study is the visibility it may bring to our new assay system. This study is being led by our own Joe Zagorski in collaboration with our clinical partners at Corewell Health.

Another exciting development is that CRIS submitted a preliminary proposal to the U.S. Food and Drug Administration to continue research should qualify our DIT in vitro assay for regulatory use to support New Approach Methodologies (NAMs). We worked closely with researchers from Health Canada, Burleson Research Technologies and the Johns Hopkins DIT working group, and our pre-proposal was selected for full proposal consideration. The full proposal was recently submitted, and we anxiously await news on whether our full proposal will be selected for funding. If so, this would provide critical resources for comparing the reproducibility of the assay across four laboratories using known reference chemicals.



A NOTE FROM THE DIRECTOR CONTINUED

We saw our membership continue to grow when we welcomed Proctor and Gamble as a CRIS partner. We continue to foster collaborations across industries to help expand the reach and impact of our work at CRIS.

We've also seen our communications impact broaden, and we have been interviewed in numerous top-tier publications, including The Washington Post, New York Times, National Geographic, and more. We are very excited about the impact of our communication efforts during this past year led by Elisabeth Anderson and the visibility it has brought to CRIS. It is simply remarkable that our weekly blogs are now averaging more than 36,500 views each month and continuing to grow.

This year, we were thrilled to welcome Kyleigh Cross as a new laboratory technician to the CRIS team. Kyleigh brings fresh energy, and her contributions have already made an impact.

These accomplishments reflect the dynamic and evolving nature of our work at CRIS. Each milestone is a testament to the hard work and dedication of our team and the support of our members and partners.

I extend my deepest gratitude to all of you for your ongoing support and participation. Your commitment to our mission is vital as we continue to advance the science of toxicology and contribute to creating a safer and healthier world.

Thank you for being an integral part of our journey. Here's to continuing our shared success in the future.

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Norbert Kaminski, Ph.D. Professor, Pharmacology & Toxicology Food and Consumer Product Ingredient Safety Endowed Chair Director, Center for Research on Ingredient Safety Director, Institute for Integrative Toxicology

Research Highlights NEW APPROACH METHODOLOGIES

Background

Historically, chemical toxicity testing has been accomplished primarily through the use of animal models. These models have proven to be costly, time-consuming, and often do not adequately mimic human bioloav. Therefore. there has been increased interest and commitment by regulatory agencies within the United States and abroad in developing in vitro human-based methodologies, known as new approach methodologies (NAMs), to address the known shortcomings of the more traditional animal models.

At CRIS, we have similarly focused on developing and utilizing human-based NAMs for our toxicity assessments of chemicals.

Currently, we are utilizing NAMs for the determination of developmental immunotoxicology testing, as well as the determination of liver toxicity, using a coculture model processing cell-type found in the liver in addition to hepatocytes.



Research Highlights NEW APPROACH METHODOLOGIES

Developmental Immunotoxicology

The many different cell types that comprise the immune system arise from the pluripotent hematopoietic stem cell (HSC). This complex process takes place early in life during fetal development and then after birth within the bone marrow.

During the past eight years, the Kaminski laboratory has utilized human umbilical cord blood-derived HSC to study the effects initially of dioxin-like compounds on HSC to B cell lineage commitment. More recently, we discovered using a technique termed singlecell RNA sequencing that in addition to the presence of lineage-committed B cells, virtually all other major white bloods, with the exception of T cells, are present in this 28-day culture system.

Under the auspices of CRIS, we are proposing to utilize this culture system as a model for screening and identifying putative developmental immunotoxicants.

Studies describing the initial use of this model system for investigating the effects of dioxinlike compounds were first published in the Journal of Immunology in 2017. The more recent characterization of this model and the development of the various cell types that comprise the immune system was published in "Blood Advances" in 2024 is currently being used for projects discussed in this Annual Report.

In this CRIS Annual Report, we discuss the application and ongoing efforts toward validation of this model for investigative and regulatory purposes.

Here at CRIS, we have similarly focused on developing and utilizing human-based NAMs for our toxicity assessments of chemicals.



Image Source: Bao et al (2019), EMBO Mol Med

Research Highlights NEW APPROACH METHODOLOGIES

Liver Co-Culture Model

One major limitation in the development and implementation of NAMS is that of metabolic activation of chemicals. Exposure to some chemicals only results in toxicity if that compound is metabolized, usually by the liver. Here at CRIS we have worked to develop NAMs with this in mind. One of such NAMs is a liver coculture model. In an organism, the liver is comprised of a complex mixture of cells, not all of which can metabolize chemicals. Therefore, we generated a model that utilized a human liver cell line (the HepaRG) which is metabolically active, and we grew this liver cell in the presence of another type of cell that can be found in the liver, the sinusoidal endothelial cell.

To model the sinusoidal endothelial cell, we utilized the SK-Hep-1 human cell line, which does have the capability to metabolize chemicals. When grown together, a chemical that requires metabolism to be toxic can be added to the system and its effects can be observed on cells that lack that capability, more closely mirroring human liver biology. This NAM was utilized in our project investigating the differential potencies of pyrrolizidine alkaloids on human endothelial cells.



Research Highlights 2023 RESEARCH

Utilizing Human Primary Leukocytes in a Metabolically Active Culture System for Assessing and Studying Immune Toxicants

As part of the ongoing efforts by CRIS to establish new approach methodologies to evaluate ingredient safety consumer products, the research team has initiated a program to expand on current capabilities in identifying and studying human immunotoxicants. As already discussed, rodents (primarily mice and rats) have served as primary animal models for establishing safe exposure levels of chemicals for humans. Due to differences in the biology across animal species, a significant effort by the Kaminski lab and more recently by CRIS, has been to establish in vitro immune function assays using human peripheral blood leukocytes for assessing the immunotoxic potential of chemicals and other agents. Although these immune function assays have largely been established and implemented for safety assessments and for elucidating mechanisms of toxicity, a major obstacle is that human leukocytes have minimal metabolic activity. Due to this, the NAMs are only useful for identifying and studying those agents that do not require metabolic activation to mediate their toxic effects, or by employing their toxic metabolites, if known and available.

Therefore, CRIS has been establishing immune function assays using a coculture approach in which human PBMCs are cocultured with the metabolically active human liver cell line, HepaRG cells, employing reference chemicals known to require metabolic activation. Using this approach, immune proliferative assays for assessing clonal expansion of activated B cells and T cells have been established as proof of principle using this coculture NAM. The goal for 2024 is to expand this coculture approach for the large number of immune function assays that have already been established without liver cells and now will be optimized for coculture with HepaRG cells. By doing so, CRIS will greatly expand the ability to identify and studying human immune toxicants in human primary leukocytes.



Research Highlights COLLABORATIVE ALLIANCES

Utilizing Developmental Immunotoxicology Assay in Real-World Application

As described earlier in this CRIS Annual Report, efforts are ongoing to qualify the human DIT assay. One avenue toward this end has been to employ this assay system for identification of putative human developmental immunotoxicants. Doing so is not a trivial task as most developmental immunotoxicants have been identified in mouse models and assumed to produce the same effects in humans, which is a big assumption. Therefore, the availability of "known" reference chemicals for assav validation is limited. One such developmental immunotoxicant identified in rodent models is lead.

In 2022, Drs. Zagorski and Kaminski submitted a grant proposal in collaboration with a clinical team in Grand Rapids, MI at the Corewell Health System proposing to recruit pregnant mothers to be screened for elevated blood lead levels and to consent to donate their umbilical cord blood hematopoietic stem cells (HSC). The grant was selected for funding.

Research is underway comparing HSC in parallel with HSC from pregnant mothers with no detectable blood lead using our DIT assay. The newborns are also followed using medical chart reviews to determine if there is evidence of altered immune competence like increased infections (e.g., ear) or evidence of allergy in association with elevated blood levels.

Mothers are being recruited from the three zip codes in Kent County, Grand Rapids, which have a very high incidence of lead poisoning. Surprisingly, in a 2016 report the Michigan Department of Health and Human Services confirmed that no Michigan county, including counties in Flint, MI, has more leadpoisoned children than Kent which County, and is localized to three zip codes.

A total of 800 mothers will be screen for blood lead levels and approximately 40 mothers with high blood lead levels and 40 mothers with low or no detectible blood lead levels (control) will be evaluated in our study.

To date we have enrolled ~300 participants and research is underway.



Research Highlights COLLABORATIVE ALLIANCE

Qualification of the CD34 Cell Developmental Immunotoxicology Assay

With the explosion of NAMs, much of the current discussion in this area has been focused on what will be required to gain broad acceptance of an in vitro assay for regulatory use. It is noteworthy that in the US, in vitro methods that are not fully "qualifited" may be fit-for-purpose for regulatory applications; however, this is determined on a case-by-case basis and is also dependent on the regulatory agency.

CRIS has been participating in a Developmental Immunotoxicology Working Group comprised of immunotoxicologists from government, industry and academia led by Dr. Fenna Sille at Johns Hopkins University to identify and qualify DIT assays.

The DIT assay developed at Michigan State University is presently the lead candidate for qualification.

Presently four laboratories have agreed to participate in an inter-laboratory validation, which will include Dr. Fenna Sille's laboratory at the Johns Hopkins, Burleson Research Technologies Inc, Health Canada and CRIS at Michigan State University.

In 2023, the four laboratories submitted a proposal to the U.S. Food and Drug Administration (FDA) seeking funding to further continue research to conduct cross comparison of the assay.

The proposal was selected for submission of full proposal to the FDA and we will have more information in 2024 on the proposal outcome.



Research Highlights 2023 PUBLICATION

CBD Liver Toxicity 2023 Publication (2022 Submission)

Evaluation of the Potential Hepatotoxicity of CBD using a Human Hepatocyte Spheroid Model.

Abstract

The United States Food and Drug Administration recently approved the use of cannabis sativa derived cannabidiol (CBD) in the treatment of Dravet Syndrome and Lennox-Gastaut Syndrome, under the trade name, Epidiolex. In double-blinded, placebo-controlled clinical trials, elevated ALT levels were observed in some patients, but these findings could not be uncoupled from the confounds of potential drug-drug interactions with co-administration of valproate and clobazam.

Given the uncertainty of the potential hepatatoxic effects of CBD, the objective of the present study was to determine a point of departure for CBD, using human HepaRG spheroid cultures, followed transcriptomic benchmark dose analysis. bv Treatment of HepaRG spheroids with CBD for 24 and 72 hours, resulted in EC50 concentrations for cytotoxicity of 86.27 μM and 58.04 μM, respectively. Subsequent transcriptomic analysis at these timepoints demonstrated little alteration of pathway data sets at а CBD gene and concentration at or below 10 µM. Although this current analysis was conducted using liver cells, interestingly the findings at 72 hours post CBD treatment showed suppression of many genes more commonly associated with immune regulation.



Indeed, the immune system is a well-established target for CBD based on immune function assays. Collectively, in the present studies a point of departure was derived using transcriptomic changes produced by CBD in a human cell-based model system, which has been shown to accurately translate to human hepatotoxicity modeling.

CRIS Activities MAINTAINING SCIENTIFIC RIGOR

Response to the European Food Safety Authority on Bisphenol A (BPA)

"When the weight of evidence does not weigh enough: EFSA's draft scientific opinion on BPA"

Abstract

In November of 2021, the European Food Safety Authority (EFSA) released a draft scientific opinion on bisphenol A (BPA) exposure and health outcomes released to the public. EFSA concluded that the most sensitive outcome category to BPA exposure is the immune system. In this scientific opinion, EFSA utilized a weight of evidence approach to conclude that it is likely that BPA exposure promotes the development of TH17 cell-mediated atopic respiratory disease (eg, wheezing, rhinitis and asthma). Here, we present a dissenting analysis to that put forward in the draft EFSA scientific opinion and raise concerns about the studies and EFSA's interpretation of data that were used to arrive at their conclusion.

You can read our response at go.msu.edu/FmC5

Response on Titanium Dioxide Research

"Comment on Bischoff et al. The Effects of the Food Additive Titanium Dioxide (E171) on Tumor Formation and Gene Expression in the Colon of a Transgenic Mouse Model for Colorectal Cancer. Nanomaterials 2022, 12, 1256"

Overview

The publication by Bischoff et al., 2022 claims to provide evidence for a promoting effect on colorectal cancer in a mouse model by titanium dioxide (TiO2). There are many serious flaws in this publication and the reported findings do not support the authors' conclusion. In fact, the results presented support, just the opposite, a conclusion of no effect.

CRIS Activities ENGAGEMENTS: RESEARCH & OUTREACH

CRIS Annual Meeting & Science Symposium

The 2023 CRIS Science Day focused on new approach methodologies in support of weight of evidence assessments for regulatory decisions.

Featuring:

- Amber Goetz, Ph.D., Syngenta: "Implementing Modern Approaches to Testing and Evaluation into Weight-of-Evidence"
- Barbara Kaplan, Ph.D., Mississippi State University: "NAMs and In Vitro Assays for Immunotoxicity with Focus on the IL-2 Luc Assay"
- Zhichao Liu, Ph.D., Boehringer Ingelheim Pharmaceuticals, Inc.: "Generative AI Promoting New Approach Methodologies (NAMs) Development"
- Kelly Magurany, M.S., NSF International: "Applying NAMs in Risk Assessment - A Framework Approach and Case Study"
- Lindsay Marshall, Ph.D., Humane Society of the United States: "Replicating the human airways in vitro - from normal physiology to disease models for drug testing."
- M. Sue Marty, Ph.D., DOW, Inc.: "Using NAMs in a NextGen Safety Assessment for a Cosmetic Ingredient"

Watch the symposium: go.msu.edu/YmC5



CRIS Activities ENGAGEMENTS: RESEARCH & OUTREACH

Society of Toxicology

Annual Meeting 2023

Presentation and Poster Session

SOT is a scholarly and professional group of scientists committed to improving global safety and health by advancing toxicology and enhancing its impact and influence.



Dr. Norb Kaminski gave a presentation on developmental immunotoxicology (DIT).

Dr. Joe Zagorski, presented a poster on the research "Evaluation of the Potential Hepatotoxicity of CBD using a Human Hepatocyte Spheroid Model."

International Association for Food Protection



Annual Meeting 2023 Presenter and Panelist

The International Association for Food Protection (IAFP) is dedicated to safeguarding the global food supply. It comprises a diverse membership including educators, government officials, microbiologists, and food industry executives. These professionals

collaborate in all facets of the food production process, from farming to preparation and represents over 50 countries.

Dr. Joe Zagorski was invited to speak as a panelist in the session "How I Learned to Stop Worrying and Love Food Chemicals: Hot Topics in Chemical Food Safety." He also participated in the PFAS conversation round table.

Communication Highlights

COMMUNICATION REACH

The Toxicology Forum-2023 Winter Meeting

Where Do Discrepancies Lie? From Safety Assessments to Media Headlines

The Toxicology Forum seeks to be the foremost platform among influential stakeholders for deliberative dialogue, which shapes decision-making and outcomes on critical issues in toxicology and its applications.

"Where Do Discrepancies Lie? From Safety Assessments to Media Headlines," focused on the session, will explore the challenges and strategies of effective science communication, highlighting how discrepancies in safety assessments and regulatory viewpoints can hinder public trust and impact regulations.

Global Alliance for Rapid Diagnostics

"Social Media for Scientists: Science Communication for the Web

The Global Alliance for Rapid Diagnostics (GARD) is a unique community of innovators, and a multidisciplinary-multicultural peer-to-peer network of equals committed to one vision: support sustainable health through early diagnosis for resource-limited populations worldwide.

"Social Media for Scientists: Science Communication for the Web" focused on empowering and equipping international scientists and researchers with the tools and knowledge to effectively share their findings and insights on various social media platforms.

Association of Science Communicators

Science Talk '23, "Social Listening for Science Communicators: Staying Ahead of the Curve"

The Association of Science Communicators (ASC) is a professional organization growing to meet the needs of the multifaceted science communication community with the goal to better address the challenges facing our society.

The ASC members voted to include the workshop "Social Listening for Science Communicators: Staying Ahead of the Curve" in SciTalk '23.







Communications Highlights COMMUNICATION REACH

Google Search Placement

Top Ranking

Our commitment to consistent and organic growth has been reflected in the growing popularity of our community-focused blog posts, many of which have reached the top search engine results. Our approach is based on using social listening to help shape our post ideas and to ensure that we meet our audience where they are, rather than where we assume they should be.

As we continue to use search engine optimization and social listening to optimize our content, we have witnessed an increase in community-driven activity on our posts.

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Rinsing: Rinsing produce in warm water for at least one minute or longer can help reduce pesticide presence on produce. Soaking: Soaking produce in warm water is one of the easiest and most effective ways to remove pesticide residues. May 23, 2022



Communications Highlights

CRIS NEW HORIZONS

Artificial Intelligence Future

Sustained Relevence

As technology advances, our strategies to ensure content remains available and accessible are proving effective. Our content has even been incorporated into new Artificial Intelligence (AI) technology. Including, Google Generative Search, Bard, and Chat GPT 3.5.

We will continue to optimize our content to ensure we're using every tool possible to keep our information relevant and easy to find.



Communications Highlights

CRIS IN THE MEDIA & NEWS

Our approach to ensuring our content is universally accessible and optimized for search engines makes it easy for the media to engage with CRIS experts.

Journalists and news outlets continue to contact CRIS experts for interviews and insights on topics related to food, household items, and cosmetics affecting our society.

In 2023, we secured interviews and additional coverage in major news outlets, including the New York Times, The Wall Street Journal, The Washington Post, Slate, Discover Magazine, and National Geographic.

THE WALL STREET JOURNAL. THE WALL STREET JOURNAL. The Washington Post NATIONAL GEOGRAPHIC SLATE DISCOVER

Communications Highlights TOP BLOGS OF 2023

- 1. Preservatives, keeping our food fresh and safe
- 2. How to Series Removing Pesticide Residue
- 3. Silicon Dioxide
- 4. Sodium Aluminum Phosphate
- 5.Petroleum Jelly
- 6. What's the risk? Zinc Oxide
- 7.Food Dye
- 8. What's the risk? Titanium Dioxide
- 9. What's the risk? Talc-based Cosmetics
- 10. Expiring Products Cosmetics



CRIS EXTERNAL SCIENTIFIC ADVISORS



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Larry Lichter





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Robert Sills, DVM PhD

THANK YOU

Our heartfelt thanks go out to the devoted members. Your engagement and dedication to CRIS are truly indispensable. The perspectives and contributions you offer to our organization are deeply valued.

Your support is the cornerstone that enables us to pursue our mission of conducting research and offering insights into the safety of food and consumer product ingredients. This work aids in facilitating evidence-informed decisions for consumers, the industry, and policymakers alike.

We're excited to keep moving forward with you, working together as we aim towards creating a future that's safer and healthier for all.

The CRIS Team



