

# ISOTOPE TRACERS IN METABOLIC RESEARCH

## PRINCIPLES AND PRACTICE OF KINETIC ANALYSIS

2011 COURSE OUTLINE

**APRIL 18 – 22, 2011**

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### FACULTY

HENRI BRUNENGRABER *Course Co-Director*

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OWEN McGUINNESS

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MICHELLE PUCHOWICZ

ROBERT R. WOLFE *Course Director*

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### GENERAL INFORMATION

#### COURSE FORMAT

The course and homework problems will be run on paperless format. Participants are expected to come with a laptop computer equipped with Excel and a wireless Internet connection. All course material (including slides and problem sets) will be available for download a few days before the course starts. Registered participants will receive the link and password **by email** on April 14. Feel free to print the downloaded material. The faculty will systematically upload any new or additional material (including problems' solutions) on the course webpage [we might want to insert here the link to the course webpage].

Note that, in order to foster intellectual exchanges without fear of plagiarism, this course will have a closed meeting format, just like a Gordon Conference.

#### COURSE CONTENT

The syllabus of the course can be downloaded at:

[http://www.mmpc.org/documents/Tracers/Tracers\\_Syllabus.pdf](http://www.mmpc.org/documents/Tracers/Tracers_Syllabus.pdf)

We would like as much as possible to tailor faculty presentations to the interests of the attendees. Feel free to send us a list of topics you wish to be developed before **APRIL 3, 2011**.

Please outline these topics in as much detail as you feel comfortable. Feel free to suggest a topic not currently listed in the syllabus.

Send your suggestions to Drs. Wolfe at: [RWolfe2@uams.edu](mailto:RWolfe2@uams.edu) and Brunengraber at: [hxb8@case.edu](mailto:hxb8@case.edu)

## **PRESENTATIONS by PARTICIPANTS**

On Tuesday evening, there will be a session where 10 participants will outline their respective ongoing or planned research project involving isotopic tracers. Therefore, we invite you to make a 7-8 min. slide presentation to outline (summarize) your research project, emphasizing the protocols that use isotopes, the quantitative data you expect to obtain, and any questions you have on the validity of protocols and data interpretation. Each presentation will be followed by comments from the faculty and attendees.

Please notify Drs. Wolfe at: [RWolfe2@uams.edu](mailto:RWolfe2@uams.edu) and Brunengraber at: [hxb8@case.edu](mailto:hxb8@case.edu) by **APRIL 10** if you wish to make such a presentation and if so, what will the title be. If your presentation is not selected for the Tuesday evening session, you will have an opportunity to present it to individual faculty (see below).

## **ONE-ON-ONE MENTORING**

Participants are invited to set up 30-minutes One-on-One Mentoring/discussion sessions with any course faculty.

On **APRIL 13**, you will have access to the online appointment form *via* the following link:  
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# MONDAY, APRIL 18 MORNING

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## WELCOME

Dr. MAREN LAUGHLIN, Senior Advisor, NIDDK, NIH

## USE OF RADIOACTIVE ISOTOPES

HENRI BRUNENGRABER

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### A. LEARNING OBJECTIVES

- How does one optimize the measurement of radioactivity of compounds labeled with  $^{14}\text{C}$  or  $^3\text{H}$ .
  - How does one measure a metabolic rate using  $^{13}\text{C}$  or  $^3\text{H}$  tracers?
  - What are the limitations of the use of radioactive isotopes to measure metabolic rates?
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### B. SECTIONS

- Measurement of beta radioactivity by scintillation counting; conversion of cpm to dpm (external standards, automatic quench correction, internal standards); how does one deal with counting artifacts (quenching, chemiluminescence).
  - Principles of measurement of metabolic rates; notion of specific activity of labeled precursor; problems and solutions with variations of specific activity of precursor (how does one avoid dealing with one equation and two variables).
  - Limitations of the use of isotopes for metabolic studies; difference between transfer of label and net flux; isotopic exchanges;  
(Difference between **exchange flux** and **net flux**)
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### C. HOMEWORK BREAKOUT (SMALL GROUPS)

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# MONDAY, APRIL 18 **AFTERNOON**

## BASIC CONCEPTS IN MASS SPECTROMETRY

ROBERT R. WOLFE

### A. LEARNING OBJECTIVES

- To gain an understanding of the main mass spectrometry techniques used to investigate metabolic processes with stable isotopes.
- To become familiar with current expressions of isotopic enrichment, including Tracer:Tracee Ratio and atom (or mol) percent excess.
- To learn how to measure isotopic enrichment by mass spectrometry (basic approaches).
- To learn how to calculate isotopic enrichment using gas chromatography-mass spectrometry and LC-MS/MS.

### B. SECTIONS

#### 1. BASIC DESCRIPTION OF INSTRUMENTATION

- Isotope ratio mass spectrometry (IRMS).
- Gas chromatography mass spectrometry (GC-MS).
- Gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS).
- Liquid chromatography-mass spectrometry (LC-MS; LC-MS/MS).

#### 2. CALCULATION OF ENRICHMENT WITH IRMS

- Correction of enrichment for background enrichment.
- Tracer:Tracee Ratio (TTR) vs. Molar Percent Enrichment (MPE).
- Skew correction factor to correct for the fact that the natural distribution of mass isotopomers is the same in the sample and the background.
- Use of a standard to calculate enrichment.
- Measurement of  $^{13}\text{C}$ -enrichment after combustion.
- Effect of sample size on observed ratio.

#### 3. CALCULATION OF ENRICHMENT USING GCMS

- Definition of total ion chromatogram, mass spectrum, and selected ion monitoring (SIM).
- Identifying appropriate fragment to monitor.
- Calculation of theoretical abundance.
- Calculation of isotopic enrichment using SIM.
- Effect of skewed abundance of tracer, skew correction factor.
- Overlapping spectra correction.

- Calculation of TTR when  $TTR > 1$ .
  - i. Using multiple ions to calculate isotopic enrichment
  - ii. Using less abundant masses to measure low levels of enrichment
  - iii. Calculation of concentration by internal standard technique

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## C. HOMEWORK BREAKOUT (SMALL GROUPS)



## TUESDAY, APRIL 19 **MORNING**

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### MEASUREMENT OF METABOLIC FLUXES WITH ISOTOPIC TRACERS

ROBERT R. WOLFE

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#### A. LEARNING OBJECTIVES

- To gain a conceptual and practical understanding of calculating the rate of substrate appearance (Ra) by tracer dilution using a single pool model with radioactive and stable isotopes.
  - To understand the benefit of priming the substrate pool, how to calculate a tracer priming dose, and the limitations of the primed-constant infusion technique.
  - To understand the basic approach for calculating substrate oxidation using a metabolic tracer.
  - To understand the calculation of fractional synthetic rate.
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#### B. SECTIONS

##### 1. TRACER KINETICS-SINGLE POOL MODELS

- Constant infusion of tracer.
- Influence of changes in uptake on calculation of rate of appearance.
- Calculation of Ra with a bolus injection of tracer.
- Priming the pool.
- Estimation of Ra in the non-steady state.
- Minimizing errors by curve fitting.

##### 2. INCORPORATION STUDIES

- Principles and calculation of substrate oxidation at the whole body level using tracers, including use of atom percent excess vs. Tracer:Tracee Ratio.
  - Bicarbonate recovery factor.
  - Improving the estimation of true precursor enrichment.
  - Priming the bicarbonate pool.
  - Determination of carbon dioxide production with labeled bicarbonate.
  - Problems in determining oxidation with tracers.
  - Labeled CO<sub>2</sub> reincorporation.
  - Contribution of naturally occurring <sup>13</sup>C to apparent CO<sub>2</sub> enrichment.
  - Fractional synthetic rate.
  - Synthetic rate.
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#### C. HOMEWORK BREAKOUT (SMALL GROUPS)

## TUESDAY, APRIL 19 **AFTERNOON**

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### GLUCOSE KINETICS (INCLUDING THE EUGLYCEMIC CLAMP)

**OWEN McGUINNESS**

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#### A. LEARNING OBJECTIVES

- Define the physiological correlates of glucose flux.
  - Learn best practices for experimental design optimization and data interpretation to evaluate insulin action.
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#### B. SECTIONS

1. OVERVIEW OF GLUCOSE KINETICS
    - Define steady state.
    - Define the relationship between glucose concentration and glucose mass in the body.
    - Identify sites and relative rates of glucose production and consumption and how these rates differ among species.
  2. WHAT ARE THE SOURCES OF GLUCOSE APPEARANCE?
    - Understand from a tissue point of view what production is.
    - Define the relative contribution of the liver and kidney to glucose production.
  3. HOW DO WE GET STARTED?
    - Choosing a tracer.
    - Understand how the sites of sampling and infusion can influence the measured rates of glucose flux.
    - Know how to optimize the study design to maximize steady state conditions.
  4. ASSESSING INSULIN ACTION:
    - Know how fasting status influences insulin action differently in mice and humans.
    - Define what insulin action is in the liver and the periphery.
    - Understand what a euglycemic hyperinsulinemic clamp is and how to deal with variable rates of endogenous insulin and glucagon secretion.
    - How to recognize and deal with tracer/model assumption errors (non steady state and negative endogenous production rates).
    - Be able to evaluate data used to calculate hepatic and peripheral insulin action.
    - Understand the principles used in assessing tissue specific glucose uptake.
  5. DATA PRESENTATION:
    - Know what information to include in a manuscript so as to adequately interpret the data.
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#### C. HOMEWORK BREAKOUT (SMALL GROUPS)

## TUESDAY, APRIL 19 **AFTERNOON** CONT'D

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### LIPID METABOLISM: BASIC KINETICS

ROBERT R. **WOLFE**, BETTINA **MITTENDORFER**

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#### A. LEARNING OBJECTIVES

- To understand the principles and limitations of various types of measurements of metabolic rates using stable isotopes.
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#### B. SECTIONS

1. LIPOLYSIS AND FATTY ACID RELEASE
  - Pros and cons of glycerol as a tracer to measure lipolysis.
  - Measurement of fatty acid flux with labeled fatty acid tracers.
  - Triglyceride-fatty acid substrate cycling limitations.
2. FATTY ACID OXIDATION
  - Possible pathways of fatty acid oxidation.
  - Citric acid cycle exchange reactions.
  - Acetate correction factor.
  - *in vivo* assessment of CPT activity.
3. TECHNIQUES FOR INVESTIGATING LIPOPROTEIN METABOLISM

**TUESDAY, APRIL 19** **EVENING**

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**INTRODUCTION TO THE NIH GRANTS PROCESS**

**DR. MAREN LAUGHLIN, SENIOR ADVISOR (NIDDK, NIH)**

- **PRESENTATIONS BY PARTICIPANTS (10)**

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# WEDNESDAY, APRIL 20 **MORNING**

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## MEASUREMENTS OF ENERGY EXPENDITURE

STEPHEN PREVIS

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### A. LEARNING OBJECTIVES

- List different methods for quantifying energy expenditure (or CO<sub>2</sub> production)
  - Identify the pros/cons for each.
  - Outline the general principle of using doubly-labeled water, listing important criteria for the experimentalist.
  - Explain the rationale for different data normalization/interpretation.
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### B. SECTIONS

1. OVERVIEW OF ENERGY EXPENDITURE
  - Where does “energy” go?
2. HOW DO I QUANTIFY TISSUE-SPECIFIC RATES OF CO<sub>2</sub> PRODUCTION?
  - Arterio-venous balance is required
  - Single vs. multiple compartments
  - Concerns about mixing/complete perfusion
3. HOW DO I QUANTIFY SUBSTRATE-SPECIFIC RATES OF CO<sub>2</sub> PRODUCTION?
  - Measure the production of <sup>13</sup>C-labeled CO<sub>2</sub>
  - Concerns about the recovery of a labeled substrate
4. HOW DO I QUANTIFY TOTAL BODY CO<sub>2</sub> PRODUCTION?
  - Direct calorimetry
  - Indirect calorimetry
    - i. Direct measurements of gas exchange
    - ii. Indirect measurements of gas exchange (ie: doubly labeled water)
5. HOW DO I PROCESS THE DATA AND NORMALIZE THE RESULTS?

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# USE OF $^2\text{H}_2\text{O}$ FOR MEASURING SYNTHESIS OF PROTEINS, FATS, STEROLS, GLUCOSE AND NUCLEIC ACIDS

## PART I.

STEPHEN PREVIS

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### A. LEARNING OBJECTIVES

- Explain the general use of precursor:product labeling ratios in biochemical research, list general equations for calculating rates of synthesis in short-term vs. long-term studies, i.e. those that run over several hours vs. those that run over several days, respectively.
- Suggest reasons that make  $^2\text{H}_2\text{O}$  a unique tracer for measuring the synthesis of various macromolecules.
- Explain why one requires knowledge of the labeling of specific hydrogen(s) in a product molecule in order to accurately determine its rate of synthesis.
- Contrast the pros/cons of using GC-MS vs. NMR to measure the labeling of molecule.

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### B. SECTIONS

1. WHAT CAN ONE DO WITH  $^2\text{H}_2\text{O}$  THAT CANNOT BE DONE WITH OTHER TRACERS?
  - Simultaneous tracing of multiple processes
2. CHOICE BETWEEN ACUTE AND CHRONIC LABELING STUDIES?
  - Source(s) of blood glucose (acute)
  - Total triglyceride dynamics (acute and chronic)
  - Protein synthesis (acute and chronic)
    - i. Single vs. multiple proteins
    - ii.  $^2\text{H}_2\text{O}$  vs.  $\text{H}_2^{18}\text{O}$
3. COMPLEMENTARY APPROACH TO GLUCOSE-INSULIN CLAMPING
  - Measurements of flux during metabolic steady-state vs “tolerance” testing

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### C. HOMEWORK BREAKOUT (SMALL GROUPS)

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## WEDNESDAY, APRIL 20 **AFTERNOON**

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### MEASURING SYNTHESIS OF ADENINE NUCLEOTIDES, COENZYME A, AND NUCLEIC ACIDS

#### **PART II.**

HENRI BRUNENGRABER, JOANNE KELLEHER, ROBERT R. WOLFE

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#### **A. LEARNING OBJECTIVES**

- Identify problems associated with the use of isotopic tracers for very long experiments (weeks or months).
  - Long-term isotopic experiments occur in an open biological system where unlabeled foodstuffs enter the system continuously.
  - During long-term isotopic experiments, labeled intermediates are recycled by salvaged pathways into *de novo* synthesis pathways.
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#### **B. SECTIONS**

- Comparing synthetic rates investigated with  $^2\text{H}_2\text{O}$  vs. [ $^{13}\text{C}$ ]substrates.  
(BRUNENGRABER)
- Whole pathway kinetics. Upgrading our understanding of pathway labeling.  
(KELLEHER)
- Information obtained on the scrambling of label into related pathways.  
(BRUNENGRABER)
- Measuring DNA synthesis *via* salvaged pathways.  
(WOLFE)

# METHODS IN PROTEIN METABOLISM

ROBERT R. **WOLFE**, NICOLAAS E. **DEUTZ**

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## A. LEARNING OBJECTIVES

- To understand the use of whole body protein turnover techniques
  - To learn how to calculate the rate of synthesis of individual proteins
  - To learn how to measure tissue protein and amino acid kinetics using tracers and arterial and venous catheterizations
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## B. SECTIONS

### 1. WHOLE BODY PROTEIN TURNOVER

- Catabolic and anabolic states
- Energy cost of protein synthesis
- Stochastic model of whole body protein turnover
- Comparison of tracers
- Isotopic determination of urea production
- Single amino acid tracer kinetics to calculate whole body protein turnover

### 2. MEASUREMENT OF PROTEIN FSR

- Constant tracer infusion
- Flooding dose tracer injection
- Sub-flooding dose tracer injection

### 3. METHODS TO ESTIMATE PRECURSOR ENRICHMENT FOR MEASUREMENT OF FSR 4

- Fractional breakdown rate
- Constant tracer infusion
- Bolus injection

### 4. ARTERIO-VEINOUS MODEL

- Measurement of A-V balance
- 3-pool and 4-pool models of protein kinetics and amino acid transport
- Measurement of tissue oxidation rate
- Technical aspects of performing A-V balance studies from mouse to human

**WEDNESDAY, APRIL 20** **EVENING**

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**FORMAL DINNER**



THURSDAY, APRIL 21

**MORNING**

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## USE OF POSITIONAL ISOTOPOMER ANALYSIS TO ASSESS PATHWAY FLUXES

CRAIG MALLOY, SHAWN BURGESS, GARY CLINE

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### A. LEARNING OBJECTIVES

- To understand the basic principles of magnetic resonance.
- To understand how the information content of NMR data differs from MS data.
- To understand how metabolic flux information is extracted from NMR data.
- To review common applications of NMR to metabolic flux measurements

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### B. SECTIONS

#### **PART I. NMR in tracer metabolism**

Basic principles of NMR (45 min) | BURGESS

- Measurement of fractional enrichment
- Measuring  $^{13}\text{C}$  and  $^2\text{H}$  isotopomer distributions

#### **PART II. Applications to biochemical physiology**

Calculating hepatic fluxes by multinuclear NMR (30 min) | BURGESS

- Gluconeogenesis and glycogenolysis
- Hepatic energy metabolism

Metabolic pathways in isolated cells (30 min) | CLINE

- Anaplerosis and substrate cycling
- ATP regeneration

#### **PART III. *in vivo* Applications**

Energy metabolism in muscle (30 min) | CLINE

- ATP regeneration
- TCA cycle kinetics

State of the art in metabolic MR (60 min) | MALLOY

- *in vivo* fat metabolism
- Metabolic fluxes in clinical research
- Hyperpolarization – intracellular fluxes in real time

THURSDAY, APRIL 21

**AFTERNOON**

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## USE OF MASS ISOTOPOMER DISTRIBUTION ANALYSIS

HENRI BRUNENGRABER, JOANNE KELLEHER, MICHELLE PUCHOWICZ

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### A. LEARNING OBJECTIVES

- To understand the differences between mass isotopomers and positional isotopomers (the latter will be discussed in detail on Thursday, May 7)
- To appreciate the multiple uses of mass isotopomer distribution for metabolic investigation, with the understanding that mass isotopomer distributions and positional isotopomer distributions yields complementary insights on metabolic regulation

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### B. SECTIONS

1. PUCHOWICZ: ANALYTICAL APPLICATIONS  
(i) measurement of low analyte enrichment by oligomerization of analyte; (ii) use of hexamethylene tetramine to amplify the  $^2\text{H}$ -enrichment on glucose carbons which can be converted to formaldehyde; measurement of low  $^2\text{H}$  or  $^{18}\text{O}$ -oxygen enrichment of water; (iii) measurement of low  $^2\text{H}$ -enrichment of analytes by isotope fractionation.
2. KELLEHER: ESTIMATING BIOSYNTHESIS FROM LABELING: Building a model from the ground up (syntheses of fatty acids, cholesterol, glucose, nucleic acids).
3. BRUNENGRABER: INVESTIGATIONS OF PATHWAY regulation + pathway discovery (metabolomics associated with mass isotopomer distribution)

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### C. HOMEWORK BREAKOUT (SMALL GROUPS)

- Errors in calculations of rates of glucose metabolism associated with the steady state assumption

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**THURSDAY, APRIL 21**

**EVENING**

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**DISCUSSION OF INSULIN AND GLUCOSE CLAMP**

OWEN McGUINNESS

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**FRIDAY, APRIL 22**

**MORNING**

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**INHERENTLY DIFFICULT PROBLEMS**

HENRI BRUNENGRABER AND COURSE FACULTY

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**A. LEARNING OBJECTIVE**

TO APPRECIATE LIMITATIONS ON THE USE OF ISOTOPES FOR METABOLIC STUDIES, USING EXAMPLES OF PROBLEMS WHICH HAVE CHALLENGED INVESTIGATORS FOR MANY YEARS:

- Measurement of Cori cycling.
- Measurement of fatty acid oxidation *in vivo*.
- Measurement of glucose production across a high blood flow organ (kidney intestine).
- Glyceroneogenesis.
- Measurement of coenzyme A and nucleic acid turnover with  $^2\text{H}$ -enriched water.
- Impact of secondary tracers.

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