# The role of DLD in the toxicity and resistance mechanism of phosphine gas in *Caenorhabditis elegans*


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## Background

- Fumigation of grains with phosphine gas is the most-widely used method to protect grains from insect pests [1]
- **Phosphine** is a small redox-active **metabolic toxin** for aerobically respiring organisms [2]
- Increasing number of insect pests and nematodes have developed a high tolerance against phosphine and pose a **threat to global grain supply** [1]
- The enzyme **dihydrolipoamide dehydrogenase (DLD)** is a subunit of the α-ketoglutarate dehydrogenase complex and was identified as a phosphine resistance factor [1]
- DLD is a key **regulatory enzyme** involved in energy metabolism pathways including glycolysis, TCA cycle and amino acid metabolism [2]
- A DLD protein variant with a **mutation** in the ***dld-1* gene** has been identified responsible for high-level of phosphine resistance [1]
  - Current hypothesis suggest **hypometabolism** to be a protective metabolic state against phosphine toxicity [1]

## *C.elegans* as a model organism

- In the nematode *Caenorhabditis elegans* (*C.elegans*) polymorphisim causes the mutation in the dld-1 gene of DLD [2]
- It is possible to culture it under environmentally controlled conditions and analysis of 1,000s of nematodes per condition, which minimize inter-sample differences [1]
- Genetic uniformity between strains [1]

## Objectives

1. Acquiring **gene expression** and **NMR-based metabolomics data** in response to phosphine exposure of wild-type *C.elegans* and the *C.elegans dld-1(wr5)* mutant generated using CRISPR gene editing
2. Comparing the strains and identifying constitutive differences between phosphine resistant and susceptible *C.elegans* strain

## Study design and data generation

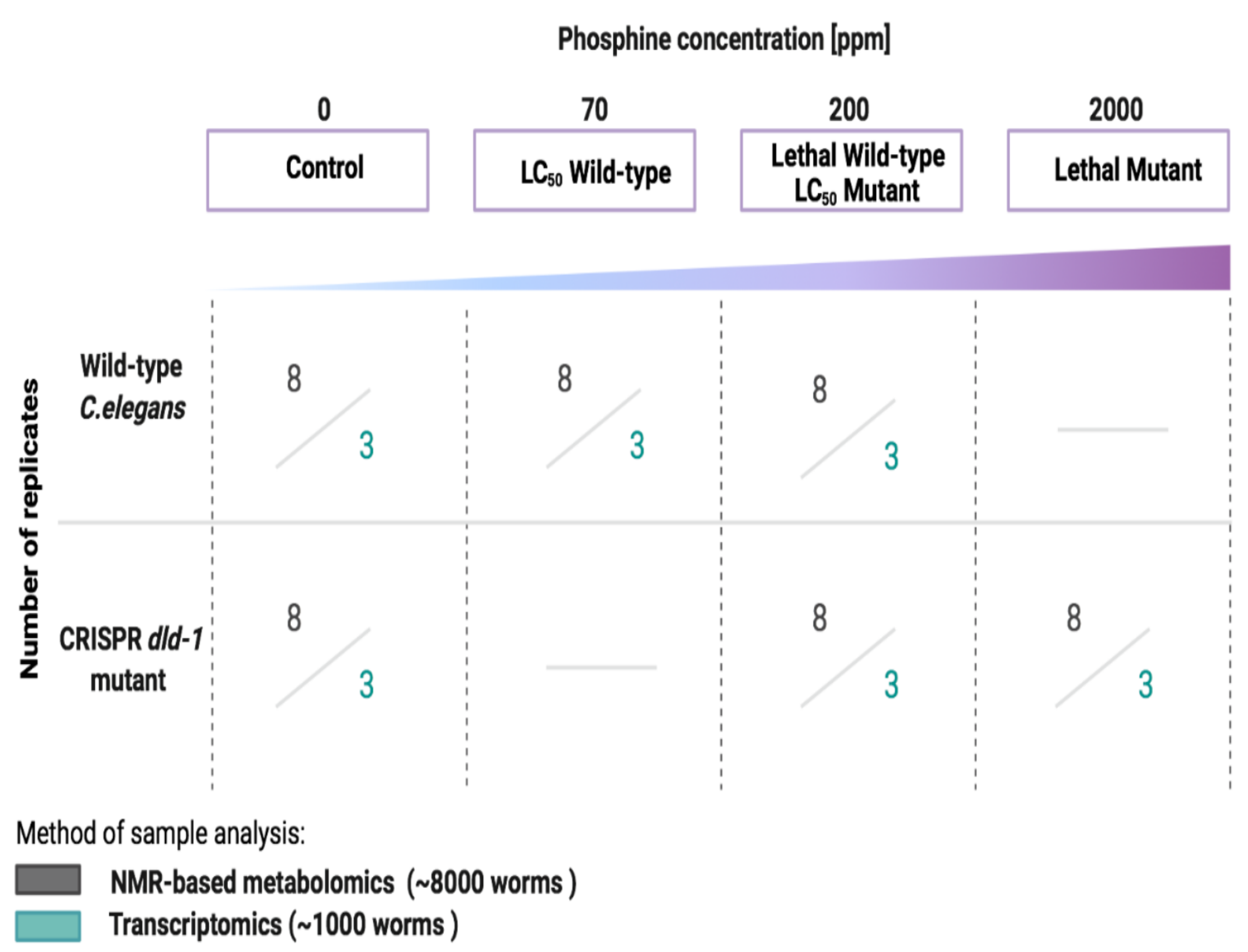


Figure 1: Study design and data generation for the analysis of wild-type *C.elegans* and phosphine-resistant CRISPR mutant strain *dld-1(wr5).* Both strains were exposed with the respective sub-lethal, lethal phosphine concentration and treatment at air (controls) for 4 hours. For NMR-based-metabolomics 8 biological replicates (each replicate with ~8000 worms) and 3 biological replicates for gene expression analysis (each replicate with ~1000 worms) were prepared. CRISPR: clustered regularly interspaced short palindromic repeats; $LC_{50}$: sub-lethal concentration; NMR: nuclear magnetic resonance.

## Data analysis and biological interpretation pipeline

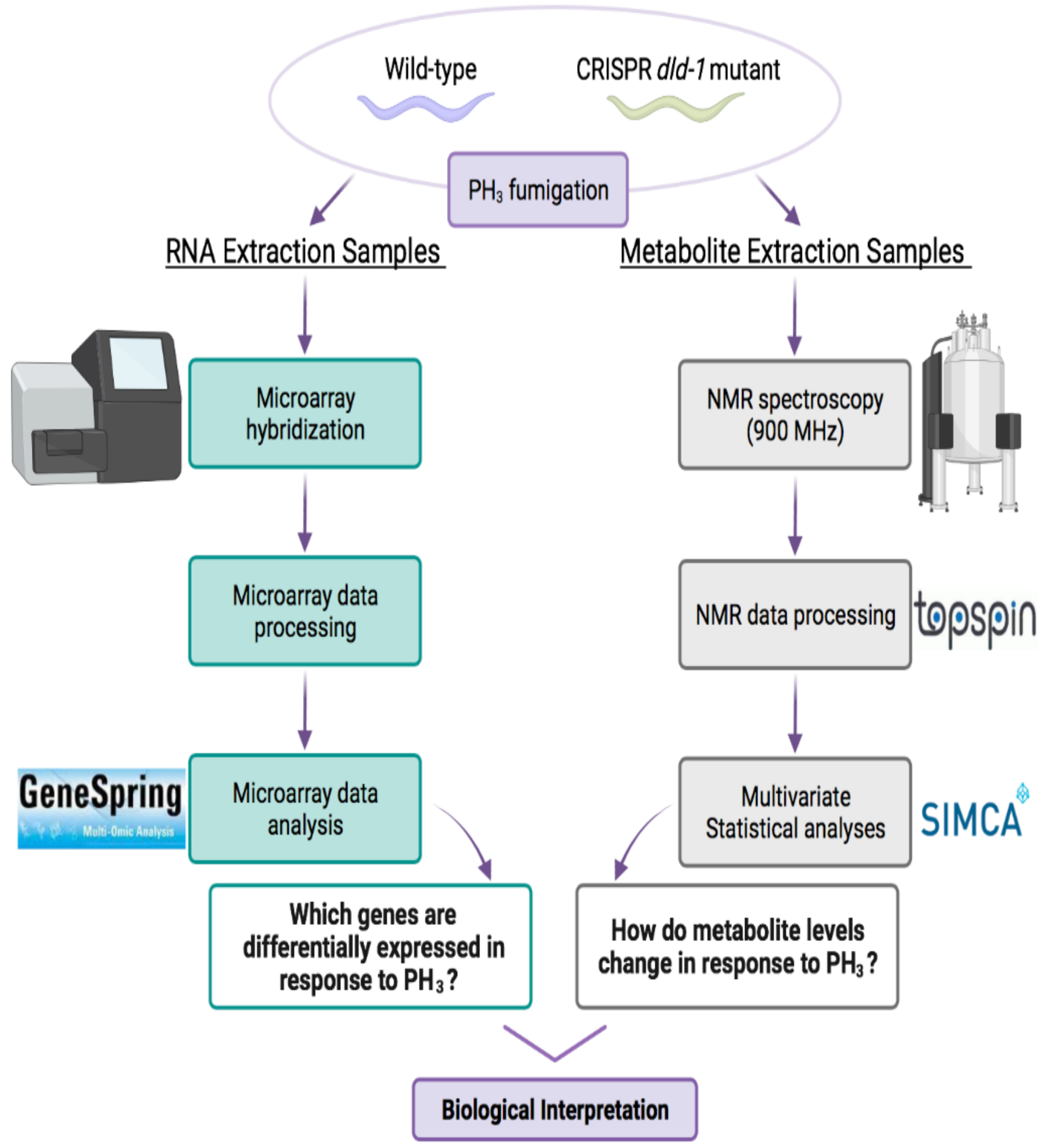


Figure 2: Data analysis pipeline for NMR-based metabolomics and microarray data. After phosphine fumigation, *C.elegans* samples were processed for RNA or metabolite extraction. NMR spectra were acquired on a 900 MHz $^1$H-NMR spectrometer, processed in TopSopin and multivariate statistical analysis is planned to be performed in SIMCA. For gene expression analysis, RNA extraction samples will be sent to to the Institute of Molecular Bioscience (IMB) Microarray Facility (Brisbane, Australia) for hybridization and data analysis will be performed in GeneSpring. The results of both approaches, transciptomics and metabolomics, will be combined for biologial interpretation.

## Methods

Two methods were conducted to correlate the response of the two *C.elegans strain* to phosphine gas to the identified genes and the metabolic pathway. The aim is to integrate gene expression and metabolite data.

**NMR-based metabolomics**
- In order to identify changes in metabolite levels and building a metabolic network to understand underlying biological process of resistance

**Microarray analysis**
- Determining differential expressed genes between the two strains, who could contribute to the phosphine resistance mechanism

## Outlook

System Biology analysis of the two *C.elegans* strains, combining transcriptomics and metabolomics data, could show further insight to in the function of DLD in the phosphine resistance mechanism and metabolic toxicity of phosphine gas. The results will show if there is a correlation between phosphine sensitivity, differential expression of genes and metabolite levels.

## References


1. Schlipalius *et al.*: A core metabolic enzyme mediates resistance to phosphine gas. *Science* 338, 807-810 (2012).

2. Ma *et al.*: Systems Biology Analysis Using A Genome-Scale Metabolic Model Shows That Phosphine Triggers Global Metabolic Suppression In A Resistant Strain Of *C. elegans*. BioRxiv:144386 (2017).



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