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Data Article

# A proteomics dataset capturing myeloid cell responses upon cellular exposure to fungicides, adjuvants and fungicide formulations

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ABSTRACT

Dendritic cells are the sentinels of the immune system, linking the innate and adaptive immune response. Myeloid and dendritic cell models have been successfully used in *in vitro* approaches to predict adverse outcomes such as skin sensitization. We here exposed a well-characterized human dendritic cell-like cell line to agricultural chemicals, including fungicide formulations, active ingredients, adjuvants and defined mixtures for 24 h to profile induced changes on protein levels. Cell pellets were harvested and prepared for bottom-up label-free analysis with peptide separation on an EASY-nano LC system 1200 coupled online with a QExactive HF-X mass spectrometer with data-dependent acquisition (DDA). The raw data files and processed quantitative data have been deposited to ProteomeXchange with the data identification number PXD034624 and are described here. The data in this article may serve as a resource for researchers interested in e.g. human toxicology, immunology, cell biology and pharmacology.

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## Specifications Table

| | |
|---|---|
| Subject | Cell biology |
| Specific subject area | Cell-based models, dendritic cells, immunotoxicity, fungicide active ingredients, adjuvants, fungicide commercial formulations, mixtures, proteomics approach |
| Type of data | Tables<br>Mass Spectrometry raw data |
| How the data were acquired | Liquid chromatography- mass spectrometry data were generated on an EASY-nano LC system 1200 (Thermo Fisher Scientific, Germany) coupled with a QExactive HF-X mass spectrometer (Thermo Fisher Scientific, Germany) using data-dependent acquisition (DDA) in positive ion more. Peptides were separated using a 60 min gradient at a constant flow rate of 250 nL/min. A top 20 method was used for MS/MS. Xcalibur software v 3.0 (Thermo Fisher Scientific, Germany) was used to control the nLC system, the mass spectrometer and for MS data acquisition. |
| Data format | Raw |
| Description of data collection | 4 batches of a myeloid cell line were exposed to the respective test materials at distinct occasions for 24 h ("main stimulation batches") and then harvested, washed with PBS, snap-frozen in liquid nitrogen and stored at −80 °C. The test materials comprised 2 reference substances, 8 fungicide active ingredients, 8 fungicide formulations, 4 adjuvants and 12 defined mixtures (here also called mixes) of substances resembling the composition of parts of the formulations investigated (see Table 1 and Table 2, also for abbreviations). All samples included for proteomics analysis showed a relative viability of over 80% compared to control. All samples treated with PPD, DiO, Folpet, Mix 1, Mix 2, Mix 3, Mix 4, Mix 7, Mix 8, Mix 10, Mix 12, and Folpan were excluded from proteomic analysis due to high variations in viability, low event counts and/or limitations in analysis capacity. One replicate treated with Folicur Xpert was excluded after normalization (sample P150). A sample list including abbreviations can be found in Supplementary table S1.<br>A subset of the samples (BEN, POL, NND, FLU, PRO, TEB, Folicur, Shirlan, Proline, Mix 5, Mix 6, and Mix 11) and associated results including further analyses are published in another manuscript [1]. |
| Data source location | Lund University, Department of Immunotechnology, Lund<br>Sweden<br>55°42′47.4″N 13°13′05.8″E |
| Data accessibility | Repository name: ProteomeXchange Consortium<br>Data identification number: PXD034624<br>https://www.ebi.ac.uk/pride/archive/projects/PXD034624<br>http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD034624 |
| Related research article | Renato Ivan de Ávila, Sofía Carreira Santos, Valentina Siino, Fredrik Levander, Malin Lindstedt, Kathrin S. Zeller<br>Adjuvants in fungicide formulations can be skin sensitizers and cause different types of cell stress responses,<br>Toxicology Reports, Volume 9, 2022, Pages 2030–2041,<br>ISSN 2214–7500<br>https://doi.org/10.1016/j.toxrep.2022.11.004. |

## Value of the Data

- Myeloid cells are crucial in innate immune responses. We here used a myeloid cell model resembling dendritic cells, which also are vital for activating the adaptive immune response. Dendritic cells are among the first cell types being exposed to pathogens and xenobiotics that enter our body. Characterizing the cells' response on the protein level when exposed to e.g. chemicals can provide a tool to predict adverse effects and involved mechanisms of action.
- The provided data can be useful for researchers in the fields of toxicology, immunology, cell biology and pharmacology.
- The data is available for any research question where the proteomic profile of the cellular response to the indicated treatments is of interest.

## 1. Objective

In previous studies we have applied a myeloid cell model to predict and better understand skin sensitization to chemicals. Traditionally, one chemical has been assessed at a time although in real life, exposure occurs to many chemicals simultaneously. In the context of pesticides, regulation has focused on active ingredients. However, also so-called "inert" additives in the pesticide formulation could cause adverse health effects such as skin sensitization. These chemicals may be sensitizing themselves (as also observed by us [2]) or contribute to combination or "cocktail effects" [3,4]. Cocktail effects have been shown to occur in different contexts, e.g. upon exposure to a sensitizer together with an irritant or detergent [5,6]. The related research article [1] includes a subset of the here described samples while we in this work describe the extended proteomic dataset profiling changes on protein levels induced by several more fungicide formulations, their active ingredients, adjuvants/additives and defined mixtures.

## 2. Data Description

The dataset described in this article comprises proteomics data based on a dendritic cell model exposed to commercially available agricultural chemicals. The raw data is shared through the ProteomeXchange Consortium via the PRIDE [7] partner repository with the dataset identifier PXD034624, project name: Myeloid cell responses to fungicides, surfactants and fungicide formulations. We here describe how the data was obtained and provide two principal component analysis (PCA) plots visualizing the samples (Fig. 1) and batch effects (Fig. 2).

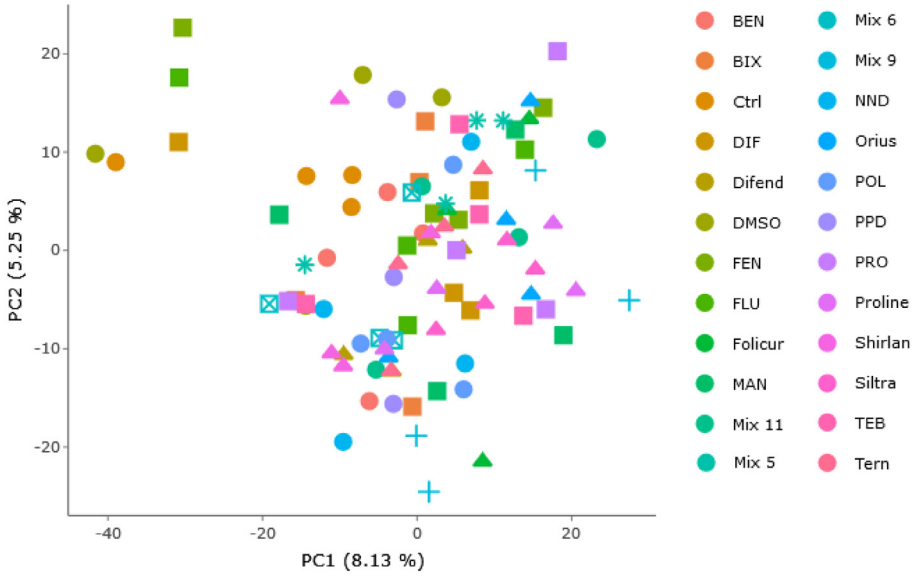


**Fig. 1.** PCA (components 1 and 2) with coloring according to test material, generated in OmicLoupe [12].

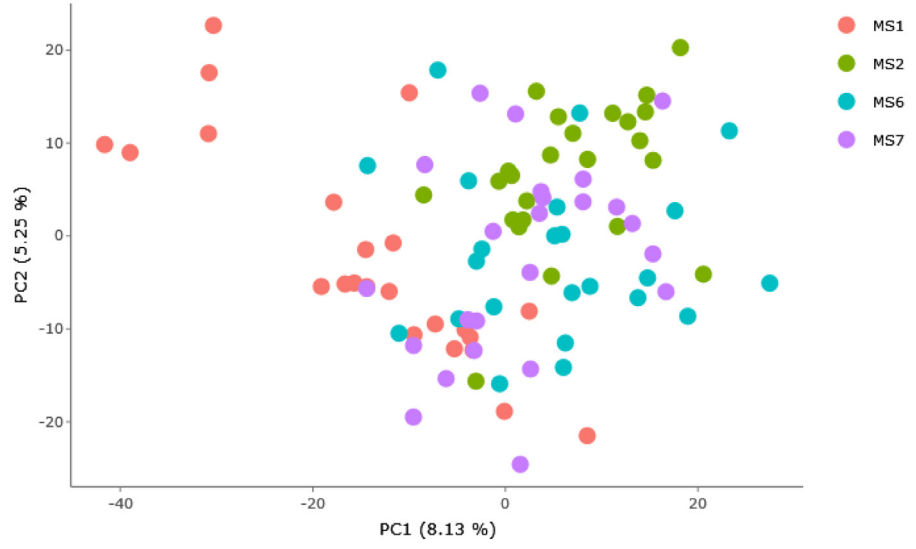


**Fig. 2.** PCA (components 1 and 2) with coloring according to main stimulation (MS) batch, generated in OmicLoupe [12].

## 3. Experimental Design, Materials and Methods

### 3.1. Cell Culture

The myeloid leukemia cell line MUTZ-3 (DSMZ, Braunschweig, Germany) was cultured in MEM-$\alpha$ medium with 20% FBS (v/v) (both from Thermo Fisher Scientific (Waltham, MA, USA)) and 40 ng/mL rhGM-CSF (PeproTech (Rocky Hill, NJ, USA)). The cells were grown in a cell incubator with humidified atmosphere at 37 °C and 5% $CO_2$ in air. Experiments were carried out with different batches of cells exhibiting a cell viability >85) and a phenotypic quality control was carried out following previously published protocols prior to each experiment [8,9].

### 3.2. Materials

The fungicide formulations were obtained from Svensk Växtskydd (Stockholm, Sweden) via the Rural Economy and Agricultural Society (Hushållningssällskap, Bjärred, Sweden). The surfactant poly(oxy-1,2-ethanediyl), alpha-sulfo-omega-[2,4,6-tris(1-phenylethyl) phenoxy]-, ammonium salt was acquired from Alfa Chemistry (Stony Brook, NY, USA) and contained 1–3 % Tristyrylphenol ethoxylate. All remaining chemicals, including agricultural ones, were obtained from Sigma-Aldrich (St. Louis, MO, USA), if no other supplier was given.

The commercial fungicide formulations tested in this study were chosen due to their frequent use in Sweden. Their active ingredients and adjuvants were acquired depending on commercial availability to investigate their toxicological effects when tested alone or in different combinations thereof (i.e. active ingredient + adjuvant). These defined mixtures mimicking a formulation were prepared based on the concentration ratios of these chemicals found in the fungicide formulation according to the supplier. If a range was indicated, the average concentration was used for calculation. The fungicide formulations were dissolved in medium, whereas other test materials were solubilized in dimethyl sulfoxide (DMSO) and then diluted in medium with a maximal DMSO concentration of 0.01% (v/v).

### 3.3. Cytotoxicity Analysis

The cytotoxicity of test materials was established according to published protocols [8,9] using Propidium Iodide staining (BD Biosciences, San Jose, CA, USA) and analyzed in a BD FACSCanto II flow cytometer. Resulting input concentrations, targeting 90% relative viability when compared to unstimulated cells ($RV_{90}$) and 500 µM for non-cytotoxic pure are summarized in Tables 1 and 2.

### 3.4. Cell Exposures

This step was performed with four different batches of cells. The protocol closely resembled published GARD® technology protocols [8,9]. In brief, 5 mL of cell suspension (in total approximately $1 \times 10^6$ cells) were exposed to the respective test materials for 24 h and then further processed as described below. Cells were harvested, washed with PBS, snap-frozen in liquid nitrogen and stored at −80 °C.

### 3.5. Protein and Peptide Extraction for Mass Spectrometry

Cell pellets were dissolved in 200 µL 5% SDS, 50 mM Tris (pH = 7.55) lysis buffer and homogenized by probe sonication with a Branson Digital Sonifier® 250-D (Branson Ultrasonics Corpo-

**Table 1**
Overview of the used chemicals and used input concentrations.

| Test material | Abbreviation | Input concentration |
|---|---|---|
| Reference controls (CAS no.) | | |
| Dimethyl sulfoxide (67-68–5) | DMSO | 0.1% (v/v) |
| *p*-Phenylenediamine (106-50–3) | PPD | 75 µM |
| Fungicide active ingredients (CAS no.) | | |
| Bixafen (581,809–46–3) | BIX | 55 µM |
| Difenoconazole (119,446–68–3) | DIF | 50 µM |
| Prothioconazole (178,928–70–6) | PRO | 115 µM |
| Tebuconazole (107,534–96–3) | TEB | 125 µM |
| Mandipropamid (374,726–62–2) | MAN | 100 µM |
| Fluazinam (79,622–59–6) | FLU | 3 µM |
| Folpet (133–07–3) | FOL | 10 µM |
| Fenpropidin (67,306–00–7) | FEN | 310 µM |
| Fungicide adjuvants (CAS no.) | | |
| Poly(oxy-1,2-ethanediyl), alpha-sulfo-omega-[2,4,6-*tris*(1-*phenylethyl*)*phenoxy*]-, ammonium salt (119,432–41–6) | POL | 500 µM |
| N,N-Dimethylcapramide (14,433–76–2) | NND | 220 µM |
| Dioctyl sulfosuccinate sodium salt (577–11–7) | DIO | 355 µM |
| 1,2-Benzisothiazol-3(2H)-one (2634–33–5) | BEN | 6.5 µM |
| Defined mixtures | | |
| DIF (35 µM) + MAN (34.5 µM) | Mix 1 | 69.5 µM |
| DIF (36.25 µM) + MAN (35.75 µM) + BEN (0.16 µM) | Mix 2 | 72.2 µM |
| FOL (10 µM) + BEN (0.049 µM) | Mix 3 | 10.05 µM |
| FLU (3 µM) + BEN (0.012 µM) | Mix 4 | 3.01 µM |
| FLU (3 µM) + POL (0.091 µM) | Mix 5 | 3.09 µM |
| FLU (3 µM) + BEN (0.0132 µM) + POL (0.0914 µM) | Mix 6 | 3.10 µM |
| PRO (86.25 µM) + NND (119.18 µM) | Mix 7 | 205.43 µM |
| BIX (18.70 µM) + PRO (74.75 µM) | Mix 8 | 93.45 µM |
| BIX (13.81 µM) + PRO (55.16 µM) + NND (24.11 µM) | Mix 9 | 93.08 µM |
| PRO (55.89 µM) + TEB (125 µM) | Mix 10 | 180.89 µM |
| PRO (28.6 µM) + TEB (63.96 µM) + NND (121 µM) | Mix 11 | 213.56 µM |
| TEB (125 µM) + DIO (13 µM) | Mix 12 | 138 µM |
| Commercial fungicide formulations (KEMI registration no.) | | |
| Difend (5233) | Difend | 256 µg/mL |
| Proline EC 250 (4688) | Proline | 58 µg/mL |
| Orius 200 EW (5540) | Orius | 148 µg/mL |
| Tern 750 EC (4371) | Tern | 26 µg/mL |
| Siltra Xpro EC 260 (5284) | Siltra | 35 µg/mL |
| Folpan 500 SC (5208) | Folpan | 3.25 µg/mL |
| Shirlan (3957) | Shirlan | 12 µg/mL |
| Folicur Xpert (5413) | Folicur | 40 µg/mL |

ration, Danbury, USA) on ice using 10% amplitude, 10 s pulse on x 5 cycles and 10 s pulse off x 5 cycles.

Samples were then centrifuged to remove debris and the supernatant was recovered. Pierce BCA protein assay kit (Thermo Fisher Scientific, Germany) was used to quantify proteins. 50 µg of protein per sample was used for hydrophilic interaction liquid chromatography (HILIC) on beads, ReSyn Biosciences, South Africa) for clean-up and automated protein digestion using a KingFisher Flex (Thermo Fisher Scientific, Germany) system in a 96-well format. The automated procedure consisted of the following steps: magnetic beads (target ratio 1:10 protein:beads) were incubated and equilibrated in equilibration buffer (15% acetonitrile (ACN)), 100 mM ammonium acetate (NH4Ac, pH=4.5). The protein samples were incubated in binding buffer (30% ACN, 200 mM NH4Ac, pH=4.5) for binding of proteins to the HILIC beads. Beads were then washed twice in 95% ACN. The beads with proteins were then incubated for 1 h at 47 °C with Trypsin (Seq grade, Promega AB) (20:1 protein:Trypsin ratio) dissolved in 50 mM ammonium bicarbonate (AMBIC).

**Table 2**
Commercial fungicide formulations tested and their composition.

| | | Composition (%, w/w) stated by the manufacturer[a] | |
|---|---|---|---|
| Product | Manufacturer | Active ingredients | Adjuvants |
| Difend | Globachem | Difenoconazole: 2.9 | NA |
| Proline EC 250 | Bayer | Protioconazole: 25 | N,N-Dimethylcapramide: >20 |
| Orius 200 EW | Nufarm Deutschland | Tebuconazole: 18–22 | Propanoic acid: 56–62; colophony: 2–5; butanedioic acid: 2–4 |
| Tern 750 EC | Syngenta Nordics | Fenpropidin: ≥70 - <90 | Solvent naphtha (petroleum): ≥2.5 - <10; poly(oxy-1,2-ethanediyl), alpha isotridecyl-omega–hydroxy-: ≥3 - <10; calcium dodecylbenzenesulphonate: ≥1 - <2.5 |
| Siltra Xpro EC 260 | Bayer | Bixafen: 5.9; Protioconazole: 19.6 | 2-[2-(1-*chlorocyclopropyl*)-2–*hydroxy*-3-*phenylpropyl*]−2,4-dihydro-1,2,4-triazole-3-thione: >0.1-<1; N,N-dimethylcapramide: ≥25; 2-etylhexanol propylen etylenglykol eter: >1-<25 |
| Folpan 500 SC | ADAMA | Folpet: 38–42 | 1,2-Benzisothiazol-3(2H)-one: <0.1 |
| Shirlan | ISK Biosciences | Fluazinam: 25–50 | 1,2-Benzisothiazol-3(2H)-one: <0.05; methenamine: 0.5–1; poly(oxy-1,2-ethanediyl), alpha-sulfo-omega-[2,4,6-*tris*(1-*phenylethyl*)*phenoxy*]-, ammonium salt: 1–5; Alkylated naphthalene sulfonate sodium salt: 3.5–5; fumaric acid: 1–1.5 |
| Folicur Xpert | Bayer | Protioconazol: 8.15; Tebuconazole: 16.3 | 2-[2-(1-*chlorocyclopropyl*)-2–*hydroxy*-3-*phenylpropyl*]−2,4-dihydro-1,2,4-triazole-3-thione: >0.1-<1; N,N-Dimethylcapramide: >20 |

[a] Some ingredients are confidential.

Peptide solutions were recovered from the plate and dried in a Speedvac (Thermo Fisher Scientific, Germany) prior to C18 desalting using BioPureSPN Mini, PROTO 300 C18 columns (The Nest Group, Inc., MA, USA). The columns were equilibrated with 100 µL 70% ACN, 5% FA, and conditioned using 100 µL 5% FA. Peptide samples were resuspended in 100 µL 5% formic acid (FA) and loaded onto the C18 column. Columns were washed with 100 µL 5% FA and peptides were eluted in 100 µL 50% ACN, and 5% FA. Eluted peptides were dried and stored at −20 °C.

### *3.6. Mass Spectrometry Analysis*

Cleaned peptide digests were resuspended and quantified using a NanoDrop 1000 (Thermo Fisher Scientific, Germany). 300 ng peptides were injected and separated using an EASY-nano LC system 1200 (Thermo Fisher Scientific, Germany) coupled with a QExactive HF-X mass spectrometer (Thermo Fisher Scientific, Germany). The analytical column was a 15 cm long fused silica capillary (75 $\mu$m* 16 cm Pico Tip Emitter, New Objective), packed in-house with C18 material ReproSil-Pur 1.9 µm (Dr. Maisch GmbH, Germany). Peptides were separated using a 60 min gradient from 5% to 90% solvent B (80% ACN, 0.1% FA) in 0.1% FA at a constant flow rate of 250 nL/min. The mass spectrometer worked using Data-Dependent Acquisition (DDA) mode in positive ion mode and acquired the full MS scan with an automatic gain control target value of $3 \times 10^6$ ions and a maximum fill time of 50 ms in a scan range from 375 to 1500 m/z. The 20 most abundant peptide ions were selected from the MS for higher energy collision-induced dis-

sociation fragmentation (collision energy: 40 V). Fragmentation was performed at 15,000 FWHM resolution with an automatic gain control target of $1 \times 10^5$ ions and a maximum injection time of 20 ms using an isolation window of 1.2 *m/z*. Xcalibur software v 3.0 (Thermo Fisher Scientific, Germany) was used to control the nLC system, the MS and to acquire the raw mass spectrometry data.

### *3.7. Mass Spectrometry Data Processing*

The raw data files were processed using MaxQuant (www.maxquant.org, version 1.6.10.43). The UniProt human proteome database as of 4th June 2020 was used as search database. Default settings were used for most MaxQuant parameters, including carbamidomethylation of cysteines as fixed modification and methionine oxidation and protein N-terminal acetylation set as variable modifications and peptide and protein group filtering at FDR $\leq$ 0.01.

The mass spectrometry proteomics data and the MaxQuant search results have been deposited to the ProteomeXchange Consortium via the PRIDE [7] partner repository with the dataset identifier PXD034624, project name: Myeloid cell responses to fungicides, surfactants and fungicide formulations.

The protein group abundance (intensity) data were further normalized using NormalyzerDE [10] with Cyclic Loess normalization [11] (Supplementary Table S2). The P150 sample (a Folicur replicate) was detected as an outlier and was excluded. A PCA plot of the sample distribution with coloring according to different test materials used is displayed in Fig. 1. As the main stimulation batch also influenced inter-sample variation, a PCA plot is also provided with coloring according to main stimulation batch in Fig. 2.

## Ethics Statements

Our work did not involve human subjects, animal experiments or data collected from social media platforms.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Myeloid cell responses to fungicides, surfactants and fungicide formulations (Original data) (PRIDE).

## CRediT Author Statement

**Renato Ivan de Ávila:** Conceptualization, Investigation, Data curation, Writing – review & editing; **Sofía Carreira Santos:** Investigation, Writing – review & editing; **Valentina Siino:** Investigation, Writing – review & editing; **Fredrik Levander:** Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing; **Malin Lindstedt:** Funding acquisition, Writing – review & editing; **Kathrin S. Zeller:** Conceptualization, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing, Investigation, Project administration.

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## Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2022.108878.

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