From Livestock Environmental Exposure to Cellular Dysfunction: Mechanistic Toxicology in Porcine Cells

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From Livestock Environmental Exposure to Cellular Dysfunction: Mechanistic Toxicology in Porcine cells

축산환경독성의 기전적 이해와 동물세포 기반 연구

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Contents

1 Environmental Exposure

Chapter 1. Background

▣ Present of pesticide study

Fungicide are widely used, but remain relatively understudied

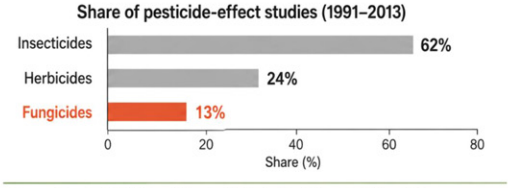
40% of total pesticide sales in the EU

Fungicides and bactericides, 2024

>90% of pesticide applications in wine-growing regions

corn | wheat | barley

Feed crops can also be associated with fungicide use.

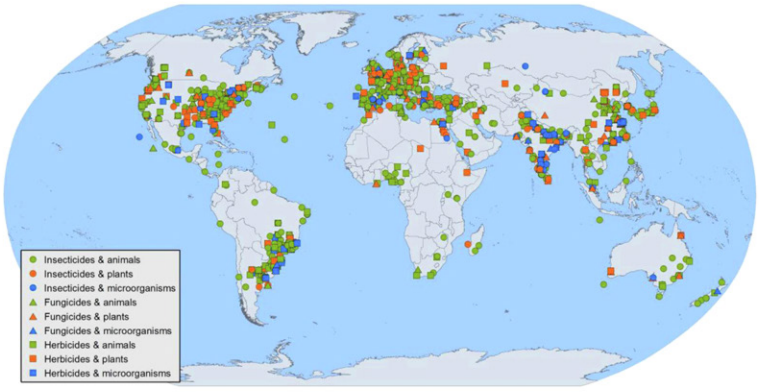


Sources: Eurostat (2024 EU pesticide sales); Zubrod et al., *Environ. Sci. Technol.* 2019.

- ✓ Fungal diseases reduce crop yield and product quality
- ✓ Fungicides are widely used in crop and feed-crop production
- ✓ Despite broad use, fungicides have received relatively limited research attention
- ✓ Fungicides should be considered an important but still underexplored class of environmental toxicants

Chapter 1. Background

▣ Negative effects of fungicide on non-target organisms

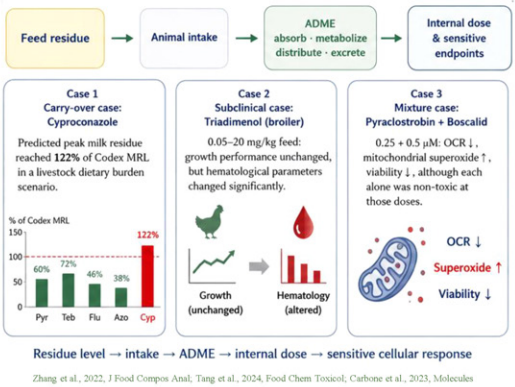


Wan, NF., Fu, L., Dainese, M. *et al.* Pesticides have negative effects on non-target organisms. *Nat Commun* **16**, 1360 (2025).

Chapter 1. Background

▣ Exposure to fungicide

Fungicide residues in livestock and biological concern



Zhang et al., 2022, J Food Compos Anal; Tang et al., 2024, Food Chem Toxicol; Carbone et al., 2023, Molecules

- MRLs manage **commodity-level residues**, but do not describe the absorbed dose, metabolites, or tissue-specific exposure.

✓ **Feed burden can shift residues into animal products;** cyproconazole was predicted to exceed the milk Codex MRL under some scenarios.

✓ **No growth effect does not mean no toxicity;** low-dose triadimenol altered hematological endpoints in broilers.

✓ **Low-dose mixture exposure can unmask toxicity;** boscalid + pyraclostrobin impaired mitochondrial function despite individually non-toxic doses.

✓ **Therefore, sensitive biological endpoints** may respond before overt production loss or carcass-residue signals are evident.

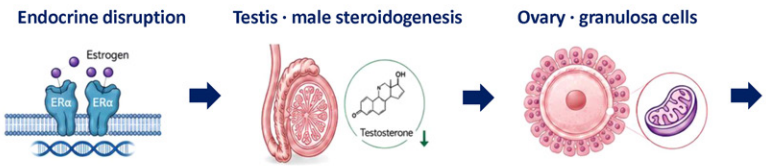
*MRL: Maximum Residue Level; ADME: absorption, distribution, metabolism and excretion

⇒ **Residue monitoring is the starting point, but biological risk should also be interpreted through ADME, low-dose repeated exposure, and mixture effects.**

Chapter 1. Background

▣ Risk of fungicide

Fungicides can disturb reproductive systems



- ✓ **Cyproconazole and epoxiconazole** may interfere with mammalian CYP-related endocrine pathways and suppress ERα-mediated signaling.
- ✓ **Prochloraz** alters fetal testis development by disrupting steroidogenesis and reducing testosterone synthesis.
- ✓ **Mancozeb** impairs oocyte quality and damages granulosa/oocyte function, partly linked to mitochondrial dysfunction.

Research perspective

: **From endocrine disruption to reproductive cellular toxicity**

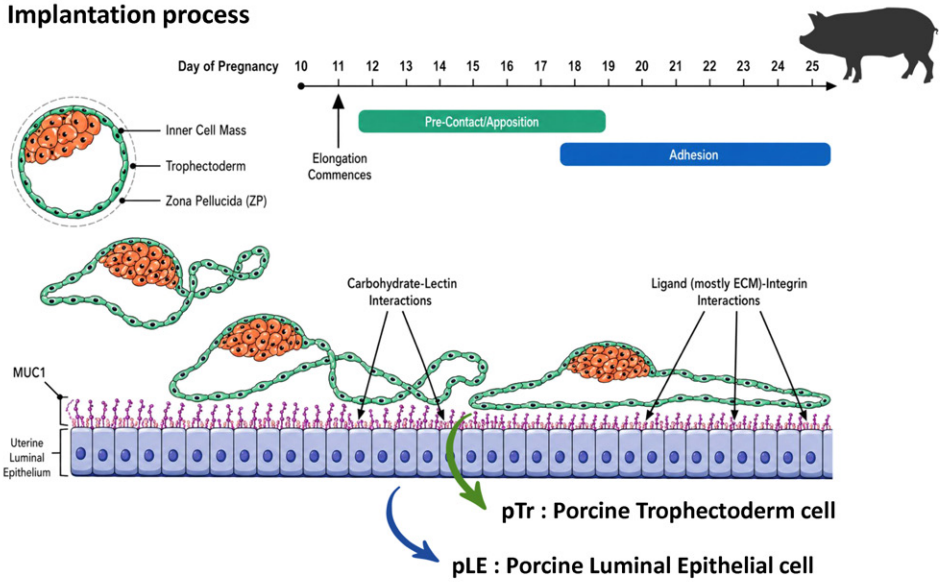
Fungicide risk is not limited to residue detection; reproductive mode-of-action evidence should also be considered.

2 Pig Endometrium and Trophectoderm

Chapter 1. Background

▣ Implantation process of pig

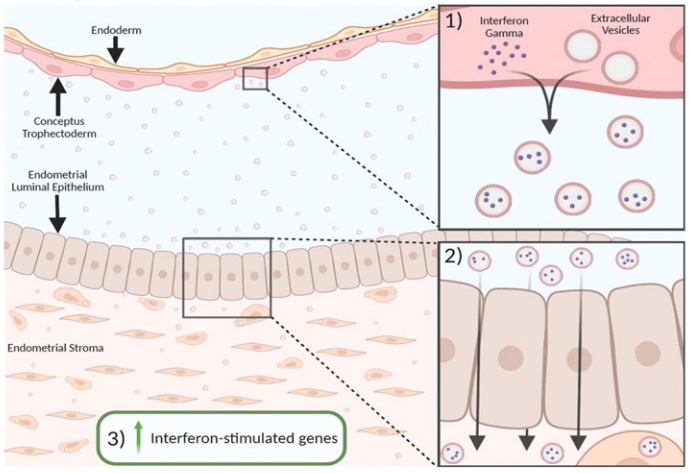
Implantation process



Chapter 1. Background

▣ Maternal-Fetal Interaction

Conceptus-maternal Interaction

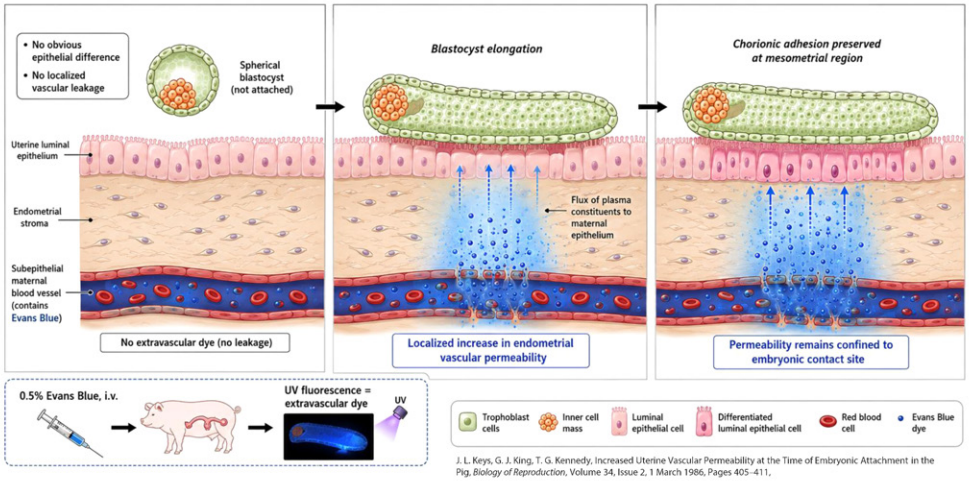


Biol Reprod, Volume 111, Issue 1, July 2024, Pages 174–185, https://doi.org/10.1093/biolre/ioae044

Chapter 1. Background

▣ Susceptibility in implantation stage

Localized increase in endometrial vascular permeability during implantation in the gilt



J. L. Keys, G. J. King, T. G. Kennedy, Increased Uterine Vascular Permeability at the Time of Embryonic Attachment in the Pig, *Biology of Reproduction*, Volume 34, Issue 2, 1 March 1986, Pages 405–411,

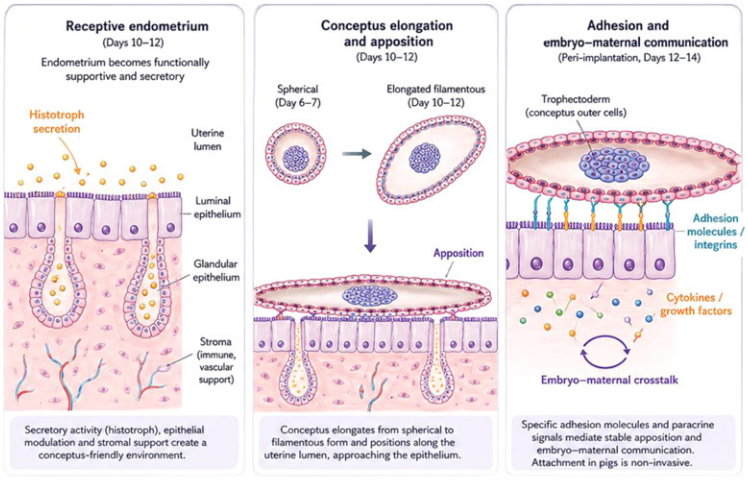
The same physiological route that supports nutrient and signal exchange during implantation may also create a window of susceptibility to circulating toxicants.

Chapter 1. Background

▣ Endometrial Receptivity

Endometrial Receptivity

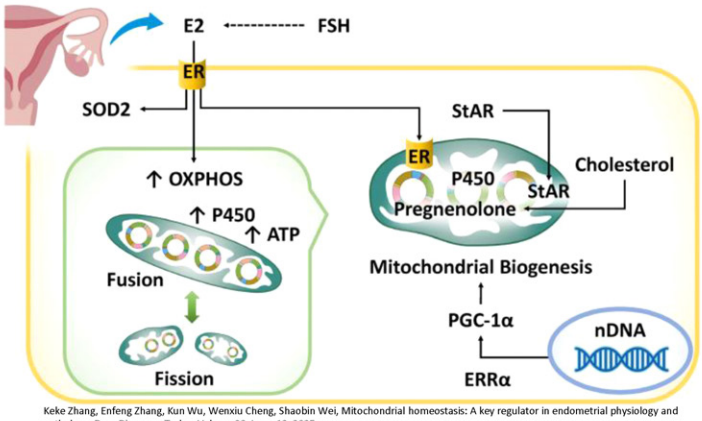
- ✓ Successful implantation also depends on whether the uterus is prepared to accept the embryo.
- ✓ Uterine receptivity is the transient state in which the endometrium becomes competent for embryo attachment and support.



Chapter 1. Background

▣ Energy Dependency of Endometrium

- ✓ The implantation window requires extensive cellular remodeling of the endometrium, including epithelial differentiation, secretory transformation, angiogenesis
- ✓ These processes are energetically supported by mitochondrial ATP production and tightly coordinated with mitochondrial Ca^{2+} handling, redox balance, and quality control.



Keke Zhang, Enfeng Zhang, Kun Wu, Wenxiu Cheng, Shaobin Wei, Mitochondrial homeostasis: A key regulator in endometrial physiology and pathology, Drug Discovery Today, Volume 30, Issue 12, 2025

- ✓ Toxicant-induced mitochondrial dysfunction may therefore compromise endometrial receptivity before overt tissue-level reproductive failure occurs.

3 Experimental Results

Study 1.

▣ Experimental architecture

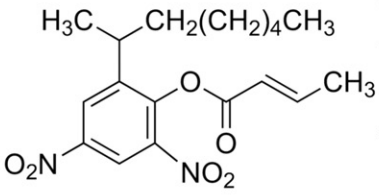
Experimental architecture

Three fungicides, one mechanistic question: how is implantation-cell function disrupted?

Study 1 Meptyldinocap	Study 2 Propiconazole	Study 3 Tolylfluanid
Model pLE / pTr	**Model** pLE	**Model** Mouse
Mechanistic focus Mitochondrial uncoupling Ca^{2+} imbalance ER stress	**Mechanistic focus** Azole fungicide mitochondrial / endocrine-related signals cellular dysfunction	**Mechanistic focus** ETC-related mitochondrial stress implantation / placental outcomes *in vivo* validation

Study 1.

▣ Meptyldinocap

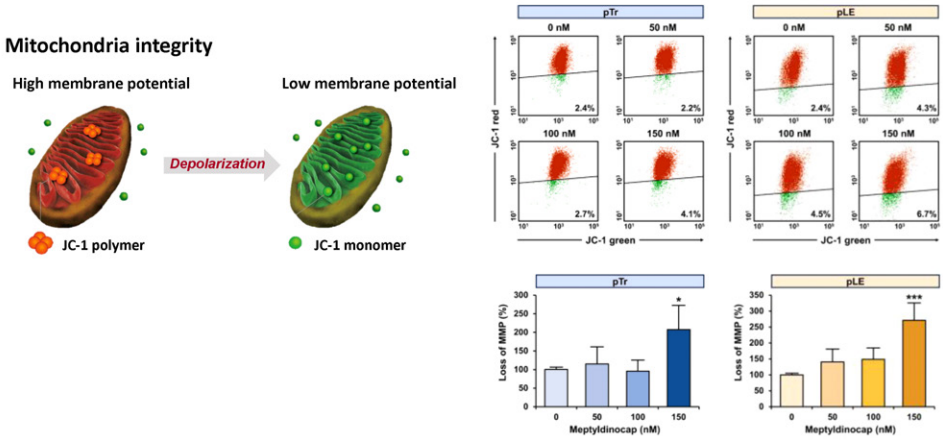


- Classification : Dinitrophenol fungicide
- Mechanisms : Meptyldinocap inhibits fungal respiration by uncoupling oxidative phosphorylation
- Target : Meptyldinocap has been used to control powdery mildew

- In South Tyrol, meptyldinocaps were detected in many public places. A maximum meptyldinocap level of 0.15 mg/kg was detected in grass samples
- In Japan, dinitrophenol compounds have been detected in 177 river-water samples, indicating a widespread exposure to the environment

Study 1.

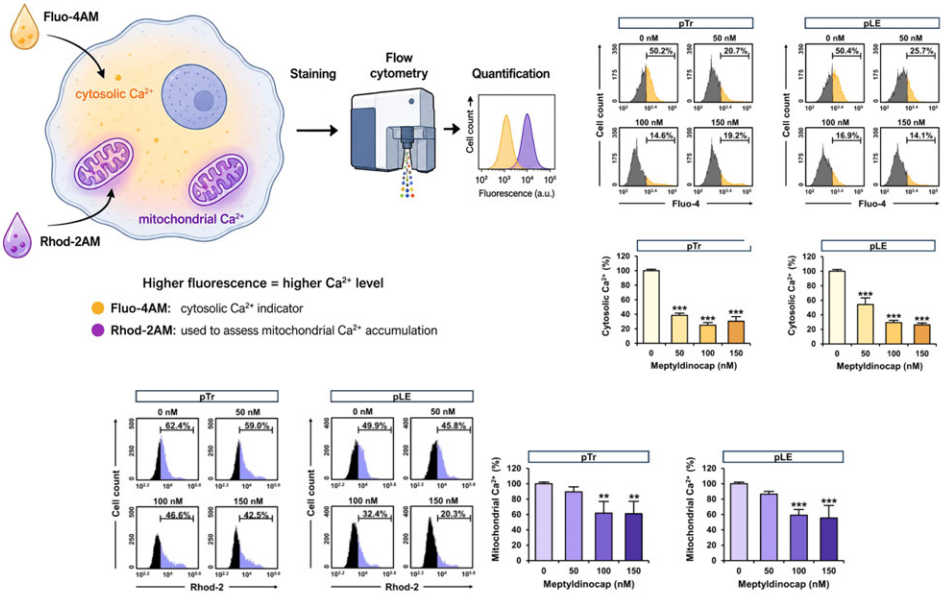
▣ Result 1 Meptyldinocap induces mitochondrial membrane depolarization in pTr and pLE cells



✓ JC-1 analysis showed increased MMP loss in both pTr and pLE cells, indicating early mitochondrial membrane destabilization.

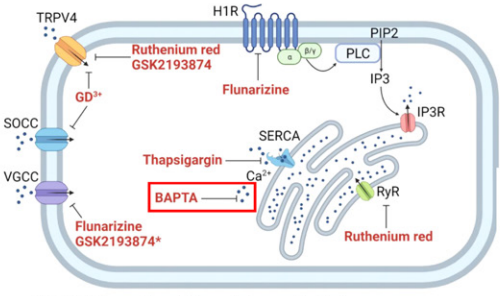
Study 1.

▣ Result 2 Mitochondrial disturbance is accompanied by Ca^{2+} depletion

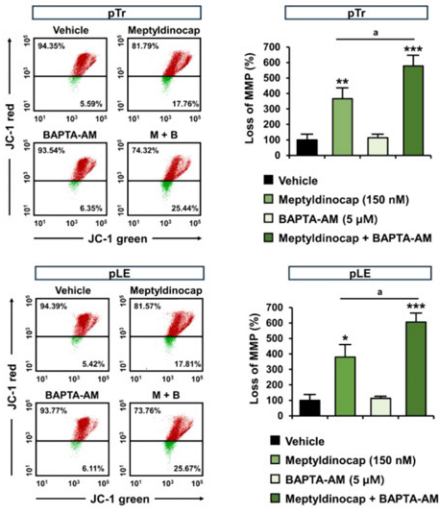


Study 1.

▣ Result 3 Calcium depletion aggravates meptyldinocap-induced mitochondrial depolarization



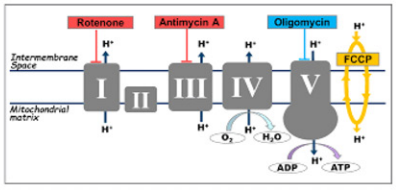
*BAPTA : cytosolic calcium chelator

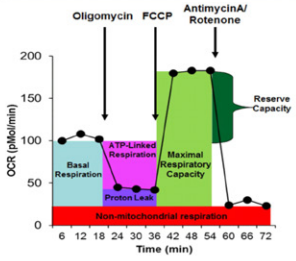


✓ BAPTA-AM further increased MMP loss under meptyldinocap exposure, linking Ca^{2+} depletion to mitochondrial depolarization.

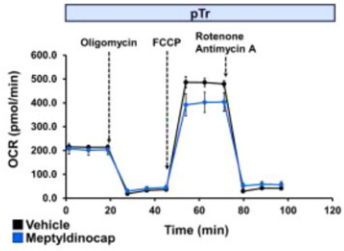
Study 1.

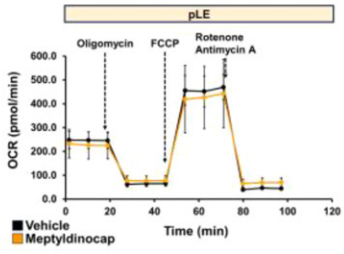
▣ Result 4 Meptyldinocap compromises mitochondrial respiratory capacity, particularly in pTr cells





*OCR : Oxygen consumption rate



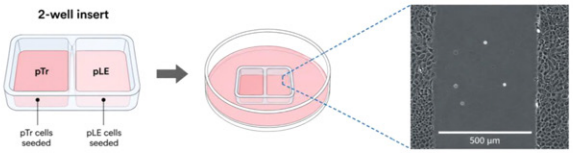


✓ OCR analysis showed reduced maximal respiration in pTr cells

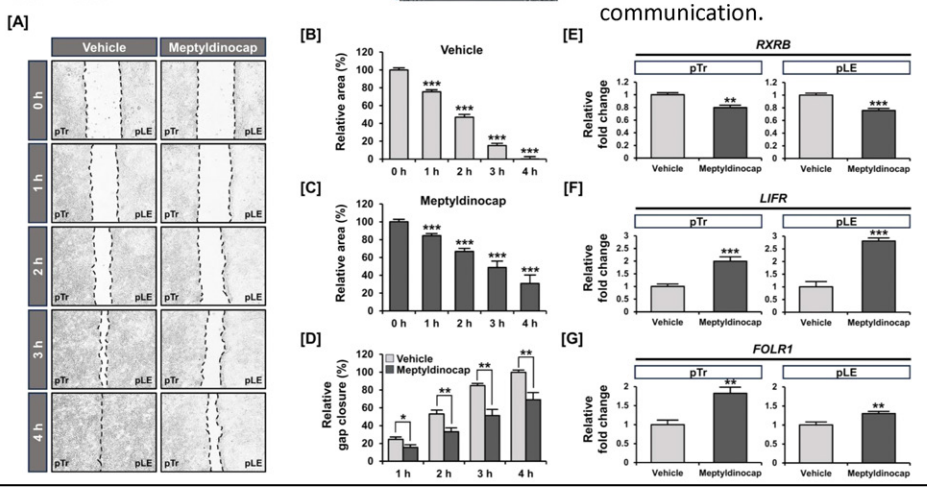
Study 1.

▣ Result 5

Mitochondrial and Ca^{2+} disruption translates into impaired pTr–pLE interaction

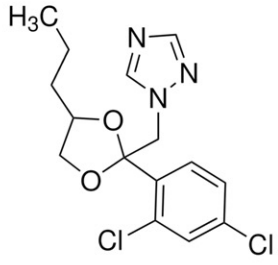


✓ Meptyldinocap delayed gap closure between pTr and pLE cells and altered pregnancy-related genes, suggesting impaired implantation-associated communication.



Study 2.

▣ Propiconazole



- Classification : Triazole fungicide; DMI/SBI Class I
- Mechanisms : Propiconazole inhibits fungal C14-demethylase (CYP51/ERG11), blocking ergosterol biosynthesis and disrupting membrane integrity
- Target : Broad-spectrum control of foliar fungal diseases, including rusts, powdery mildew, leaf spots,

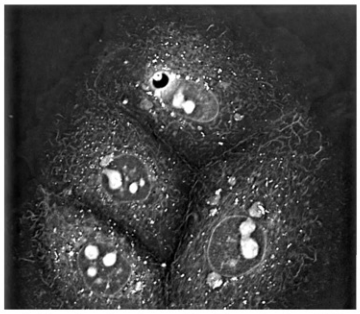
- In U.S. streams, propiconazole was detected in 17% of 103 samples from 29 streams in 13 states; detected fungicide levels ranged from 0.002 to 1.15 μg/L
- In Belgium, propiconazole was detected in surface-water samples from 16 locations and in wastewater-treatment-plant effluents, with reported levels of 1.9–178.3 ng/L
- In Korea, residues were monitored in greenhouse-grown cabbage, shallot, and spinach; initial residues were 1.46–6.26 mg/kg and dissipated by 90–99% within 14 days

Study 2.

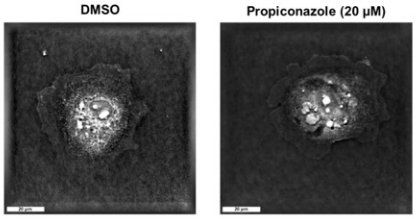
▣ Result 1 Propiconazole reduced mitochondrial contents and activity in pLE cells

Live-cell imaging and Mito Tracker staining indicate reduced mitochondrial abundance and activity after propiconazole exposure

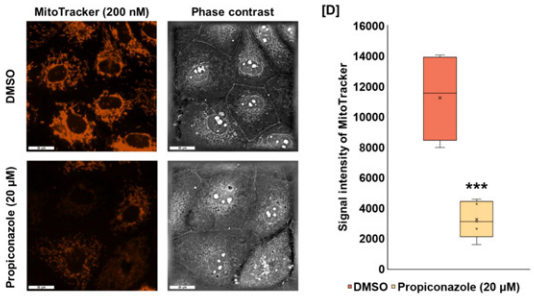
✓ **Representative live-cell image**



Label-free imaging shows abundant mitochondrial structure in untreated pLE cells



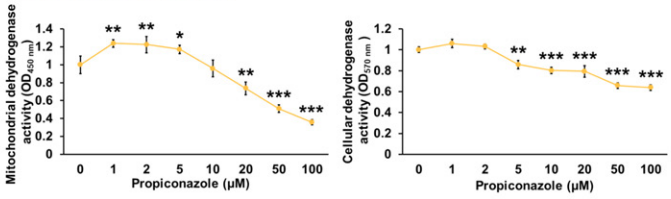
✓ **MitoTracker Fluorescence assay**



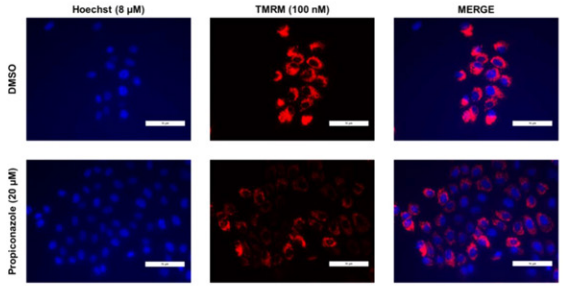
Study 2.

▣ Result 2 Propiconazole impaired mitochondrial membrane potential in pLE cells

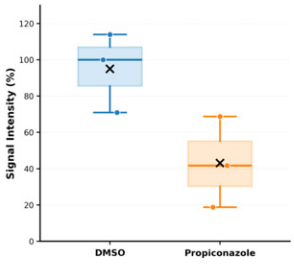
✓ **Metabolic enzyme activity**



✓ **TMRM-based $\Psi\Delta_m$ detection**



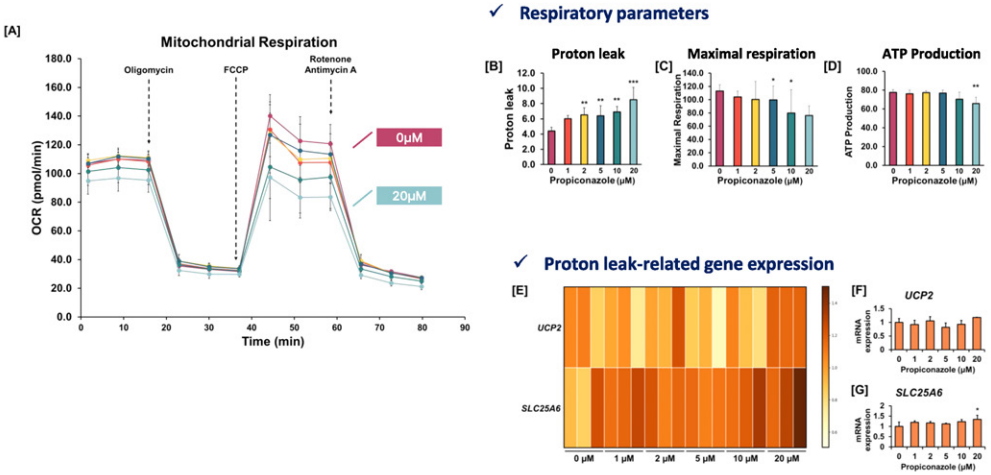
✓ **TMRM intensity**



⇒ **TMRM accumulates in polarized mitochondria; reduced red fluorescence reflets MMP depolariztion**

Study 2.

▣ Result 3 Propiconazole disrupts mitochondrial respiration and increases proton leak

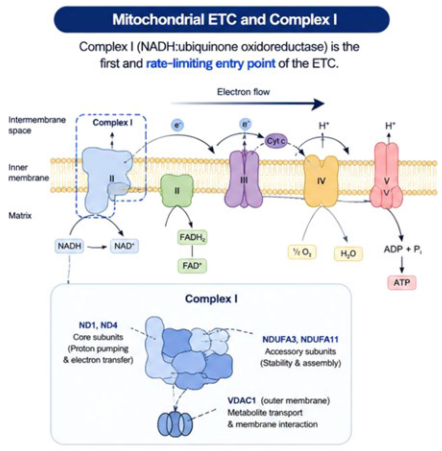


⇒ **Propiconazole impairs mitochondrial respiration in pLE cells, characterized by increased proton leak and reduced respiratory efficiency**

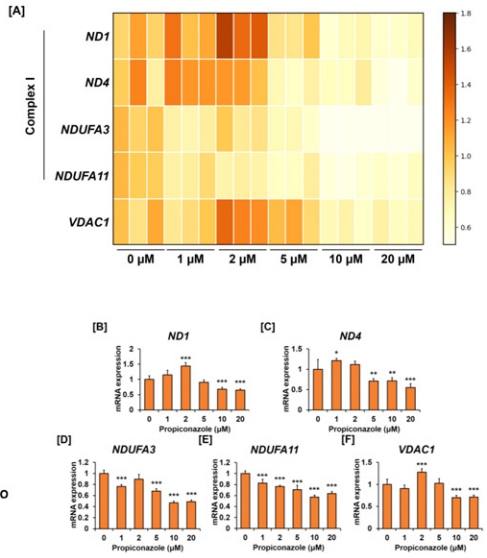
Study 2.

▣ Result 4 Propiconazole downregulated mitochondrial Complex I-related genes

Heatmap and qPCR analyses showed dose-dependent suppression of Complex 1-associated transcripts, suggesting impaired mitochondrial electron transport



✓ Reduced expression of complex1-related genes may contribute to mitochondrial dysfunction in pLE cells exposed to propiconazole

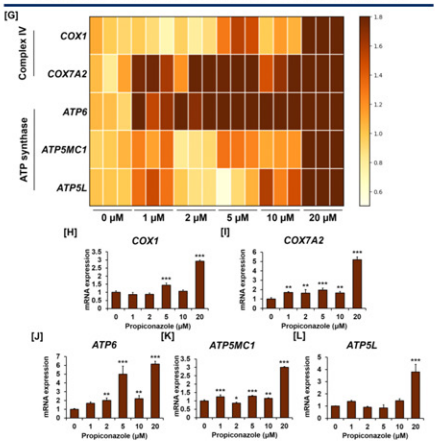


Study 2.

▣ Result 5 pLE cells exhibit transcriptional adaptive responses to impaired OXPHOS

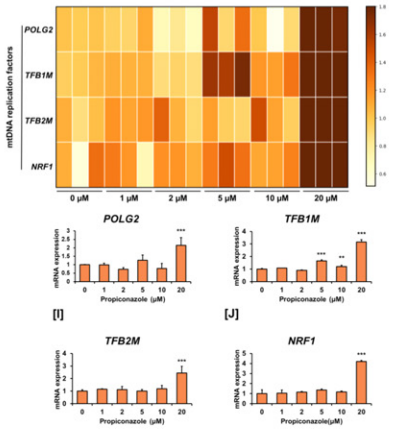
OXPHOS-related and mtDNA regulatory transcripts were upregulated, suggesting a compensatory response to impaired mitochondrial respiration.

Adaptive induction of OXPHOS component genes



Genes associated with Complex IV and ATP synthase were elevated after propiconazole exposure

Upregulation of mtDNA maintenance and transcription factors

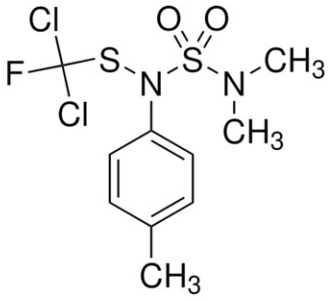


Increased expression of POLG2, TFB1M, TFB2M, and NRF1 suggests activation of mitochondrial biogenesis and mtDNA regulatory pathways.

✓ **These transcriptional changes suggest that pLE cells mount a compensatory mitochondrial stress response to propiconazole-induced OXPHOS impairment.**

Study 3.

▣ Tolylfluanid



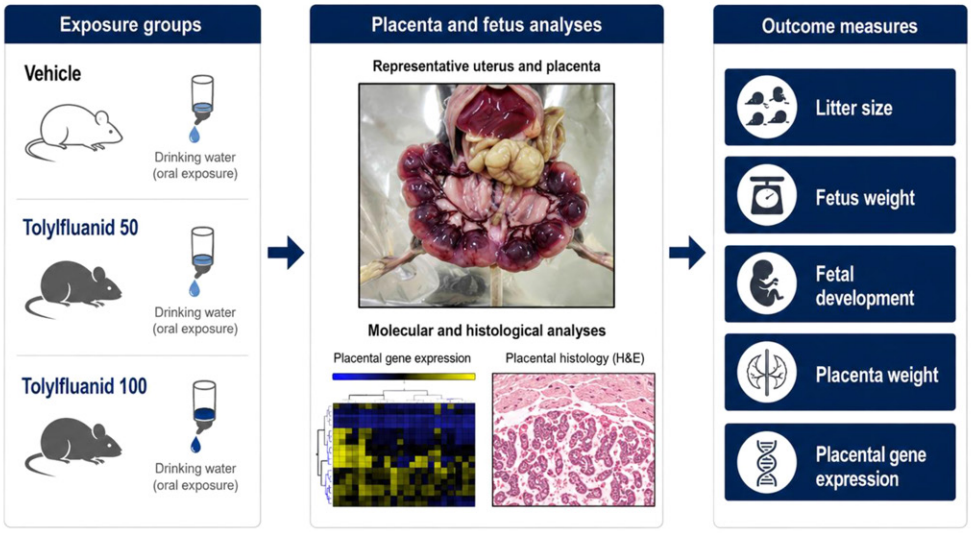
- Classification : Sulfamide fungicide
- Mechanisms : Tolylfluanid disrupts mitochondrial respiration by inhibiting enzymes in the ETC
- Target : ornamental plants against gray mold (Botrytis), against late blight and powdery mildew

- tolylfluanid and its metabolites were detected in groundwater near agricultural areas, and exposure may be possible through vegetables used with tolylfluanid
- a residual level of tolylfluanid up to 13.3 mg/kg has been confirmed in strawberries grown in a greenhouse in Norway
- the internationally estimated daily intake (IEDI) of tolylfluanid is estimated to be 0.043 mg/kg for adults and 0.048 mg/kg for children

Study 3.

▣ Tolylfluanid

Determination of effects of environmental toxicants on mouse fertility and mechanisms of fetal development and growth restriction



Analysis of pesticide effect on fetal development and litter size in mice

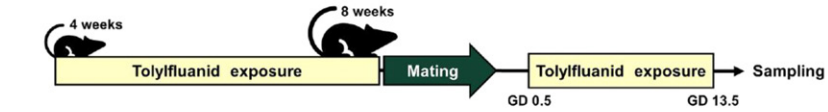
Study 3.

▣ Result 1

Maternal weight and fertility were largely unaffected by tolylfluanid exposure

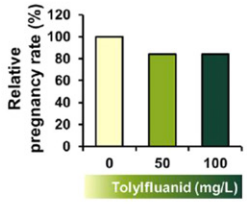
MATERNAL PHYSIOLOGY

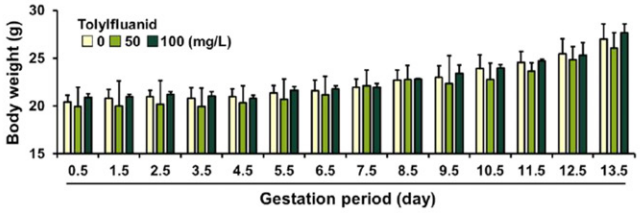
✓ Exposure across puberty and pregnancy in C57BL/6 mice



EXPOSURE GROUPS · DRINKING WATER

0 mg/L	50 mg/L	100 mg/L
vehicle (0.1% DMSO)	low dose	high dose

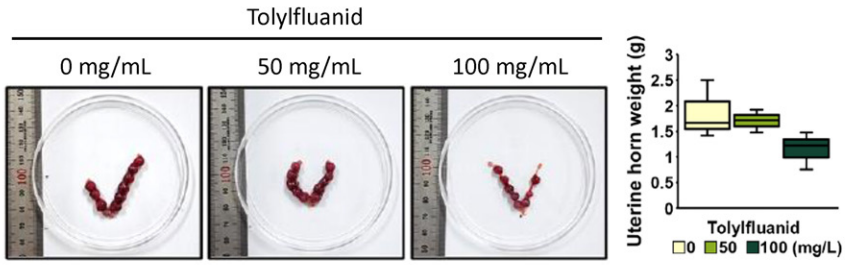




Study 3.

▣ Result 2 Underdeveloped fetuses and lighter uterine horns at 100 mg/L

GROSS MORPHOLOGY

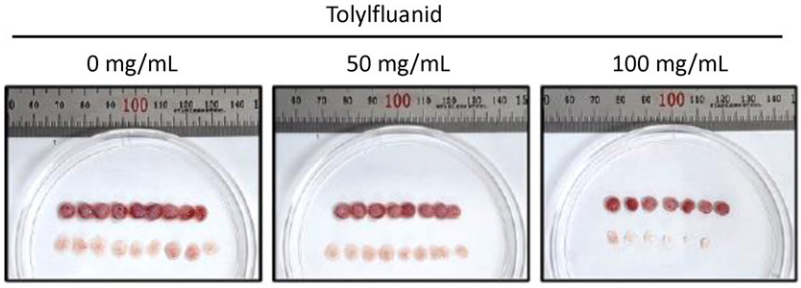


- ✓ **Uterine horn weight :** Decreasing trend at the highest dose (pregnant mice)
- ✓ **Non-pregnant mice :** Uterine horn weight significantly reduced (Suppl.)

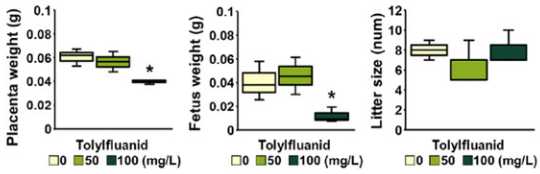
Study 3.

▣ Result 3 Significant fetal and placental growth restriction

FETAL & PLACENTAL GROWTH



- ✓ **Placenta weight ↓** significant at 100 mg/L
- ✓ **Fetus weight ↓** significant at 100 mg/L
- ✓ **Litter size** unchanged across all doses

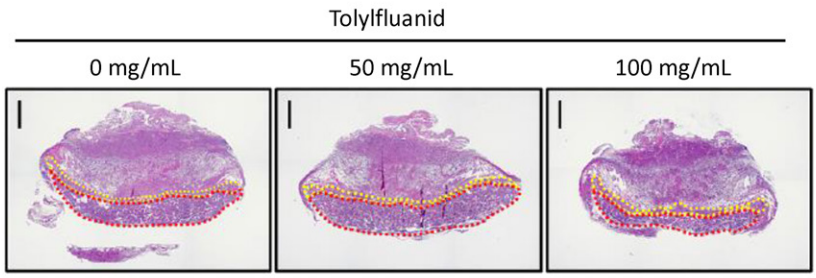


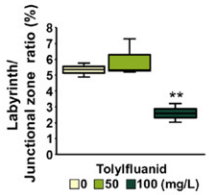
→ **Number maintained, size restricted:** a fetal growth restriction (FGR) phenotype

Study 3.

▣ Result 4 — Reduced labyrinth-to-junctional-zone ratio impairs exchange

PLACENTAL ARCHITECTURE





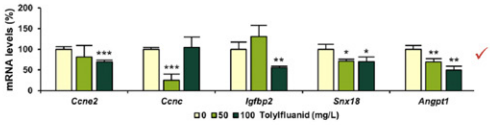
WHAT THE H&E SHOWS

- ✓ **Labyrinth / junctional zone ratio ↓** significant at 100 mg/L (**)
- ✓ **Labyrinth = exchange zone** site of maternal–fetal nutrient & gas transfer
- ✓ **Junctional zone** energy storage & hormone production

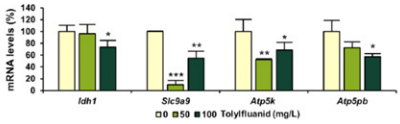
Study 3.

▣ Result 5 — Coordinated dysregulation: development ↓, mitochondria ↓, inflammation ↑

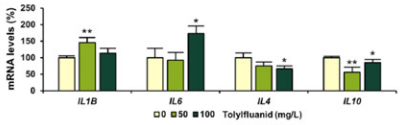
PLACENTAL GENE EXPRESSION



- ✓ **Development genes ↓**
  Ccne2, Ccnc (proliferation); Igfbp2 (growth); Angpt1 (angiogenesis); Snx18 (nutrient transport)



- ✓ **Mitochondrial genes ↓**
  Idh1, Slc9a9, Atp5k, Atp5pb



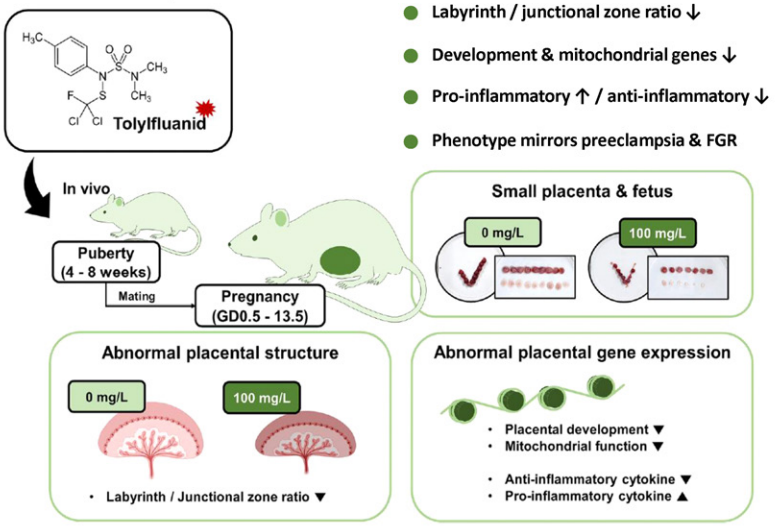
- ✓ **Inflammatory imbalance**
  pro-inflammatory Il1b, Il6 ↑ / anti-inflammatory Il4, Il10 ↓
- ✓ **Similarity with PE / FGR**
  Snx18, Idh1, Slc9a9 also fall in human preeclampsia & FGR placentas

Study 3.

▣ Tolylfluanid

Impacts of tolylfluanid on implantation and placental development: Disruption of mitochondrial function and implantation-related gene expression *in vitro* and *in vivo*

- Smaller placenta & fetus, litter size preserved
- Labyrinth / junctional zone ratio ↓
- Development & mitochondrial genes ↓
- Pro-inflammatory ↑ / anti-inflammatory ↓
- Phenotype mirrors preeclampsia & FGR

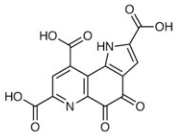


Study

▣ Ongoing study

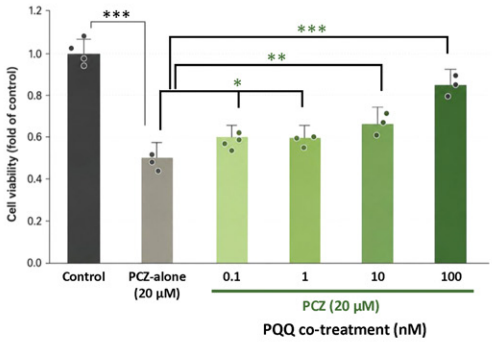
Mitochondria Enhancer

✓ Pyrroloquinoline quinone (PQQ)



- **PGC-1α pathway activation**
  promotes mitochondrial biogenesis and mtDNA regulation
- **Quinone-based redox activity**
  supports electron transfer and mitochondrial energy metabolism
- **Oxidative stress reduction**
  lowers free radicals and helps preserve ATP production

▪**Cell viability recovery effect**



▪**Transcriptional recovery effect**