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In vitro screening of azole fungicides for antiandrogenic effects – comparison with *in vivo* effects

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eSi Meeting, September 29-30 2006, Alcante, Spain

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Disposition

- Background
- Aim of the study
- Study design
- The *In Vitro* assays
- Results
- Summery
- Conclusion

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Background

- Growing concern of permanent damage to the endocrine and nervous systems after exposure to even low levels of pesticides under development.

- Azole fungicides are used in large amounts in the control of fungi in grain crops and to a lesser extent in vegetable and fruit production.

- The fungicides are relatively fat-soluble, and readily absorbed across the gastrointestinal cannel. Therefore, the public is exposed to the fungicides if residues exist in food products.

- In general, the azole fungicides have a low acute toxicity but little is known about their potential health risks at low chronic exposures.

Background

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Prochloraz has multiple mechanisms of action *in vitro*:

Aryl hydrocarbon (AhR) agonism	EC_{50} ~ 1 µM	(Long *et al.*, 2003)
Aromatase inhibition	IC_{50} = 0.3 µM	(Vinggaard *et al.*,2000)
ER antagonism	IC_{50} ~ 25 µM	(*Andersen et al.*, 2002)
AR antagonism	IC_{50} = 4 µM	(Andersen *et al.*, 2002)

Prochloraz

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Aim of the study

The results are part of a lager project, which main object is to investigate the effects of some frequently used azole fungicides on the endocrine system, including *in vitro* and *in vivo* examinations, and to assess whether *in vitro* assays can be used to predict *in vivo* effect.

Epoxiconazole

Tebuconazole

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Study design

In Vitro Screens

AR reporter gene assay and steroid synthesis testing in H295R cells

CHO or human adrenocortical carcinoma cells

In Vivo Screens

In utero and perinatal exposure

Dosing GD 7-PND 16:
Control, Epoxiconazole, Tebuconazole

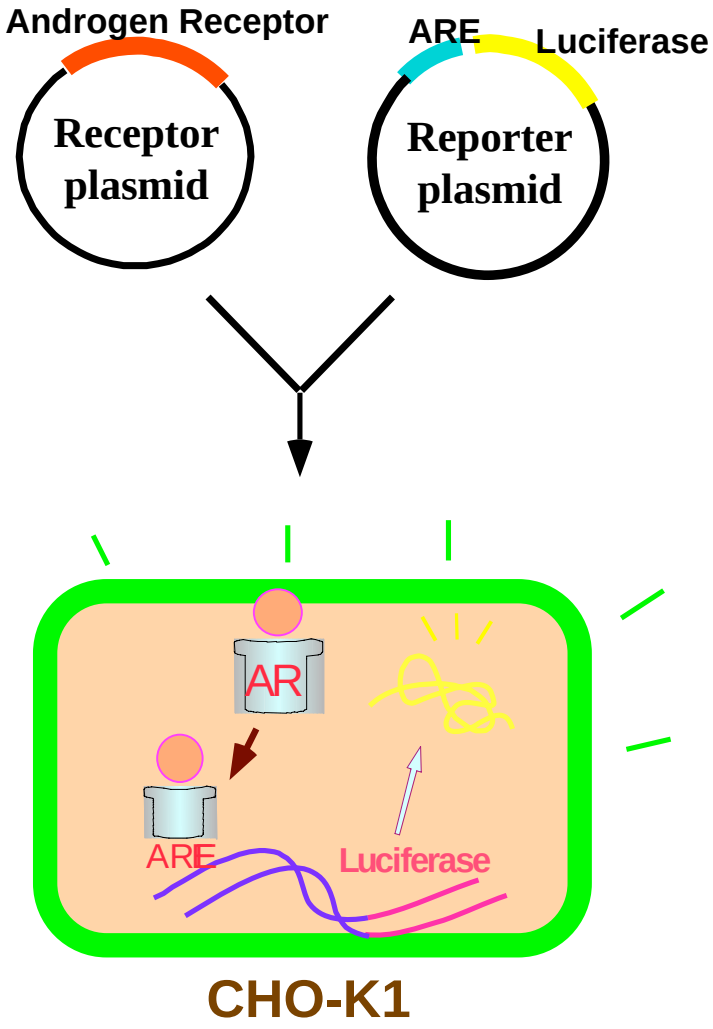
GD 7–21

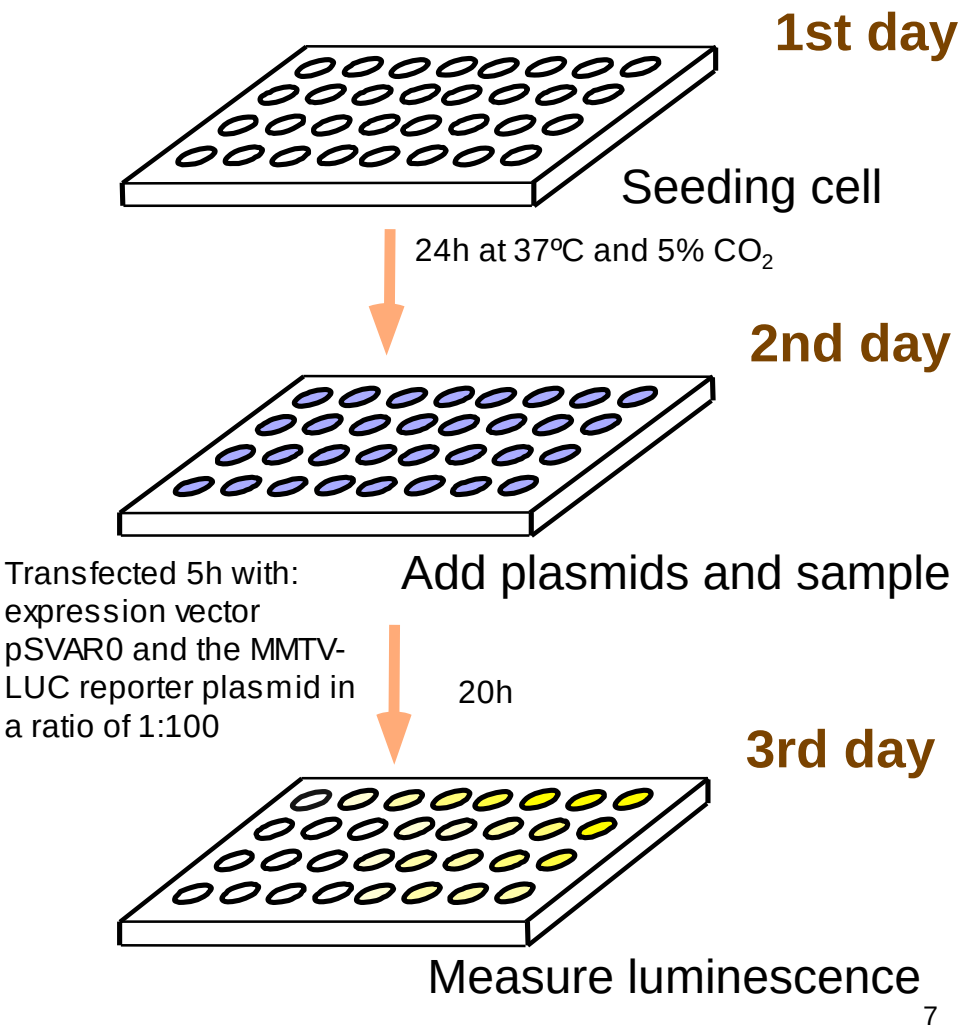
PND 1-16

Fetuses were analyzed for effects on hormone levels

Number of nipples and AGD recorded
(Typical endpoints to test for feminization of male offspring and masculinization ot the female offspring)

Assay procedure for AR reporter gene assay





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H295R cell assay - Test Design

Seeding
$2x10^5$ cells/well in 24-well culture plates

↓

Acclimatization
24h at 37ºC and 5% CO_2

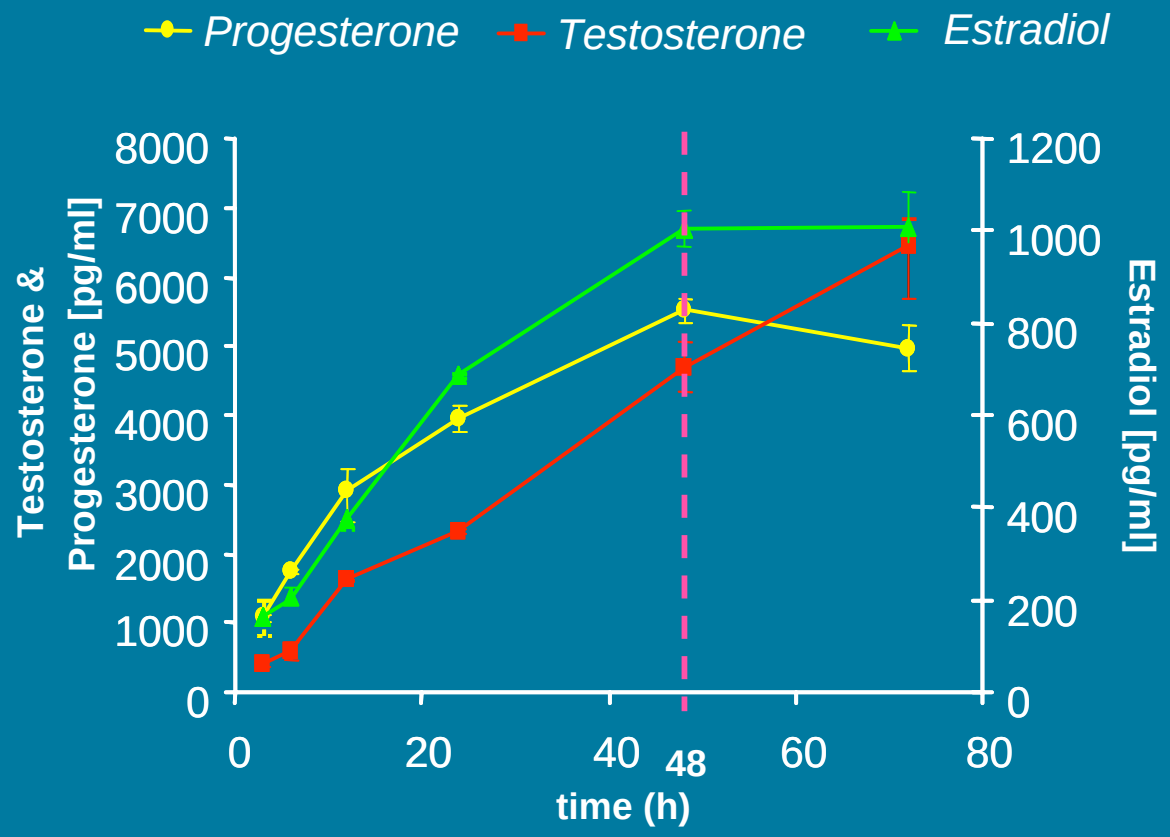
↓

Exposure
48h at 37ºC and 5% CO_2

↓

Hormone Determination
Delfia time-resolved fluorescence kit

H295R Cell Basal Hormone Production
Time Series



Results

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Steroid synthesis assay – H295R cells

Results – *In Vitro*

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The AR reporter gene assay

Androgen receptor antagonism in vitro

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Results – *In Vivo*

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Effects on offspring after perinatal exposure

Hormone data GD 21 foetus

	17α-hydroxyprogesterone (pg/testis)	Testosterone (ng/testis)	Progesterone (ng/testis)
Control	1.95±0.54 (4)	1.75±0.71 (5)	0.037±0.025 (5)
Tebuconazole 50 mg/kg	**8.39±2.59*** (7)	1.25±0.40 (7)	**0.103±0.035*** (7)
Tebuconazole 100 mg/kg	**6.59±3.88*** (9)	**0.88±0.46*** (9)	0.084±0.063 (9)
Epoxyconazole 15 mg/kg	1.76±1.36 (6)	1.62±0.59 (8)	0.029±0.019 (8)
Epoxyconazole 50 mg/kg	0.94±0.48 (13)	1.11±0.56 (20)	0.027±0.019 (20)

Data represent the mean ± SD
() = n ; * significance level P < 0.05

Results – *In Vivo*

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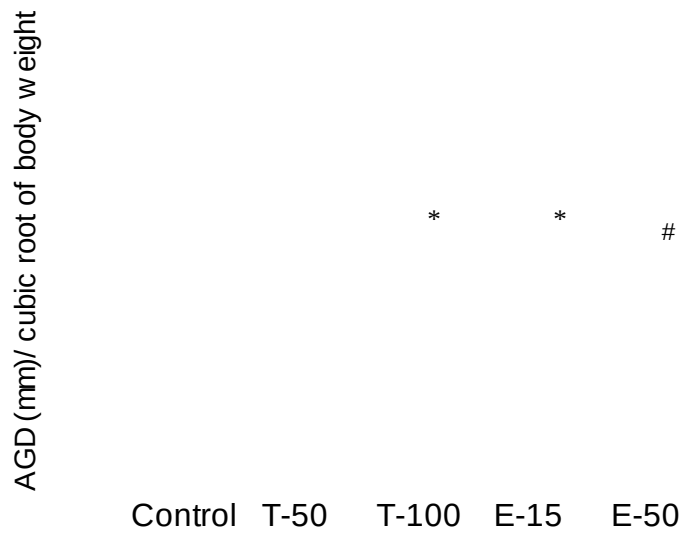
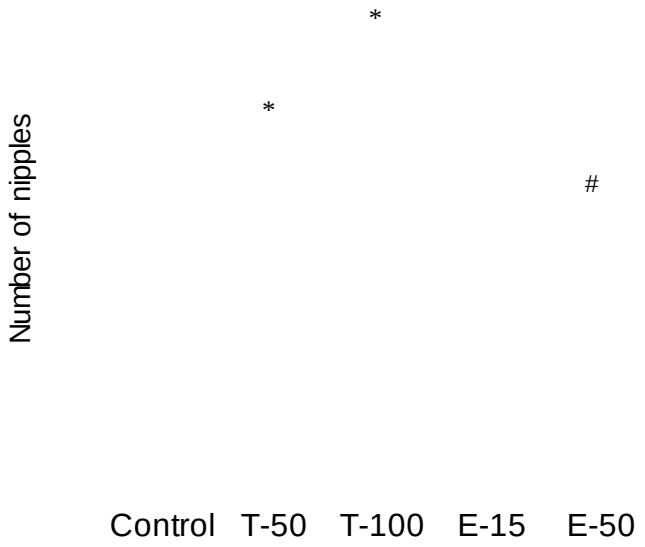
Anogenital distance PND 1



Nipple retention PND 13



only one pup

T-50: Tebuconazole 50mgkg, T-100: Tebuconazole 100mgkg, E-15: Epoxiconazole 15 mgkg, E-50: Epoxiconazole 50 mgkg

Summary

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In Vitro

- Both tebuconazole and epoxiconazole inhibited testosterone and estradiol production, and increased progesterone production

- Tebuconazole and epoxiconazole proved to be antagonists of the androgen receptor

P: Prochloraz, T: Tebuconazole, E: Epoxiconazole

	P	T	E
In vitro			
AR effects	↓	↓	↓
Steroid synthesis			
Testosterone	↓	↓	↓
Estradiol	↓	↓	↓
Progesterone	↑	↑	↑
In vivo			
AGD/cubic root of bw	↑	↑	↑
AGD/cubic root of bw male	↓	↔	↔
Nippels male	↑	↑	↔
Testosterone GD 21	↓	↓	↔
Progesterone GD 21	↑	↑	↔

In vivo

- Tebuconazole caused an increase in testicular progesterone levels and a decrease in the testosterone levels.

- Tebuconazole increased the number of nipples in the male pups and increased AGD in female pups

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Conclusions

- The results obtained *in vitro* are in good agreement with the effects observed *in vivo*.

- Tebuconazole and prochloraz showed antiandrogenic effects both *in vitro* and *in vivo*.

- Antiandrogenic effects were also seen for epoxiconazole *in vitro*, however the observed effects *in vivo* was not quite what might be predicted from the *in vitro* experiments, which can be due to the fact, that the *in vitro* screen may be more sensitive than *in vivo*.

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Thank you
for your attention!