Available online at www.sciencedirect.com
ScienceDirect
Reproductive Toxicology 22 (2006) 647–658
Reproductive Toxicology
www.elsevier.com/locate/reprotox

Effect of conazole fungicides on reproductive development in the female rat☆

John C. Rockett[a], Michael G. Narotsky[a], Kary E. Thompson[a,1], Inthirany Thillainadarajah[a], Chad R. Blystone[a,b], Amber K. Goetz[a,b], Hongzu Ren[a], Deborah S. Best[a], Rachel N. Murrell[a,b,2], Harriette P. Nichols[a], Judith E. Schmid[a], Douglas C. Wolf[a], David J. Dix[a,*]

[a] *Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, USA*
[b] *Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC 27695, USA*

Received 20 December 2005; received in revised form 27 April 2006; accepted 17 May 2006
Available online 12 June 2006

Abstract

Three triazole fungicides were evaluated for effects on female rat reproductive development. Rats were exposed via feed to propiconazole (P) (100, 500, or 2500 ppm), myclobutanil (M) (100, 500, or 2000 ppm), or triadimefon (T) (100, 500, or 1800 ppm) from gestation day 6 to postnatal day (PND) 98. Body weight (BW) and anogenital distance (AGD) at PND 0, age and BW at vaginal opening (VO), estrous cyclicity, and body and organ weight at necropsy were measured. BW at PND 0 was unaffected by treatment. AGD was increased by M2000. VO was delayed by M2000 and T1800. Estrous cyclicity was initially disrupted by P500, P2500 and T1800, but later normalized. At PND 99 there was a decrease in BW by T1800, an increase in liver weight by P2500 and T1800, and an increase in ovarian weight by M2000 and T1800. It is concluded that exposure to P, M and T adversely impacted female rodent reproductive development.
Published by Elsevier Inc.

Keywords: Myclobutanil; Propiconazole; Triadimefon; Reproduction; Development; Female; Rodent; Rat

1. Introduction

The conazoles are a large family of synthetic fungicides used extensively in agricultural applications. Some are also used pharmaceutically, primarily for the treatment of candidiasis, cryptococcosis, and coccidiomycosis. Fungicidal activity occurs through inhibiting the action of cytochrome P450 (CYP)-51 (lanosterol 14α-demethylase), which converts squalene to ergosterol [1]. CYP51 is the only P450 gene family having catalytically identical orthologues in different biological kingdoms [2–5]. In mammals, the CYP51 reaction is part of the pathway leading to the biosynthesis of cholesterol. Cholesterol is the primary sterol in the cell membrane of mammalian cells, and is also required to make sex steroid hormones [6].

Exposure of rodents to conazole fungicides has resulted in multiple toxic endpoints, including carcinogenic, neurological, reproductive, and endocrinological effects. These effects may be due to the fact that conazoles can alter the expression and activity of a number of CYP enzymes [7]. They can act as both inducers and inhibitors, dependent upon the tissue and specific conazole being considered. For example, some conazoles have been developed to selectively inhibit CYP19 aromatase, for the treatment of breast and prostate cancer [8,9].

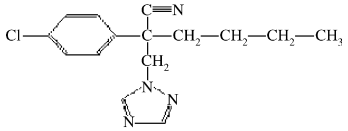
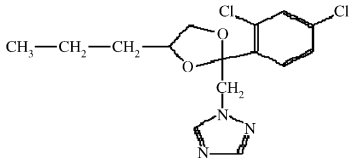
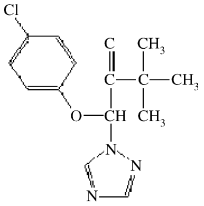
Thus, although conazoles offer extensive benefits in terms of crop protection and the treatment of human fungal infection and cancer, their ability to inhibit CYP enzymes involved in the biosynthesis of steroid hormones can potentially produce endocrine-related side effects, such as depletion of

☆ The information in this document has been funded wholly by the U.S. Environmental Protection Agency. It has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

* Corresponding author at: National Center for Computational Toxicology (D343-03), Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, USA. Tel.: +1 919 541 2701; fax: +1 919 541 1194.
E-mail address: dix.david@epa.gov (D.J. Dix).
[1] Present address: Reproductive Toxicology, Pharmaceutical Research Institute, Bristol-Myers Squibb Company, New Brunswick, NJ 08903, USA.
[2] Present address: Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, 600 Moye Boulevard, 6S-10 Brody Building, Greenville, NC 27834, USA.

0890-6238/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.reprotox.2006.05.008

Table 1
Chemical and other parameters of myclobutanil, propiconazole and triadimefon

	Myclobutanil	Propiconazole	Triadimefon
Chemical name	a,*n*-Butyl-a-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile	1-[2-(2.4-Dichlorophenyl)-4-propyl-1.3-dioxolan-2-ylmethyl]-1H-1.2.4-triazole	1-(4-Chlorophenoxy)-3.3-dimethyl-1-(1H-1.2.4-triazol-1-yl)-2-butanone)
Example trade name(s)	Nova®, Eagle®, Systhane®	Banner®, Orbit®, Tilt®	Bayleton®, Amiral ®, Sadifon®, Strike®
CAS#	8671-89-0	60207-90-1	43121-43-3
Rat acute oral LD_{50}	Females: 1620 (1270–2050) mg/kg; males: 2000 (1230–3240) mg/kg[a]	Technical: 1517 mg/kg; formulated: 1310 mg/kg[b]	Formulated: 300–600 mg/kg[c]
Molecular weight	288.81	342.25	293.78
Molecular formula	$C_{15}H_{17}ClN_4$	$C_{15}H_{17}Cl_2N_3O_2$	$C_{14}H_{16}ClN_3O_2$
Molecular structure			
1997 U.S. usage [14] (lb)	160,998	459,367	48,471
Crops (in descending order of 1997 application)	Grapes, apples, almonds, cherries, strawberries, tomatoes, peaches, nectarines, sweet peppers, water melons	Wheat, rice, pecans, peaches, sweet corn, dry beans, peanuts, barley, celery	Wheat, grapes, apples, pumpkins, sugar beets, water melons, cantaloupes, cucumbers, pears, squash

[a] http://www.hailir.com/Myclobutanil.htm.
[b] http://www.pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/propiconazole-ext.html.
[c] http://www.extoxnet.orst.edu/pips/triadime.htm.

testosterone [10] and an increased risk of adverse effects during pregnancy [11–13]. Despite their widespread usage [14] and a general tendency towards the inhibition of CYP function, the amount of *in vivo* triazole data available in the scientific press is rather limited. Summary information from rodent registration studies can be obtained from Integrated Risk Information System (IRIS) and other TOXNET databases (http://www.toxnet.nlm.nih.gov/index.html). However, a detailed examination of the effects on female reproductive development has not been previously reported for the three conazoles examined herein (triadimefon, myclobutanil, and propiconazole, see Table 1 for further details). The current study was thus conducted under the hypothesis that developmental (prenatal and postnatal) exposure to each of the three selected triazole conazoles would adversely and similarly impact female reproductive development in rats.

2. Methods and materials

2.1. Animal husbandry

All aspects of animal care, handling and treatment were conducted in EPA facilities certified by the American Association for Accreditation of Laboratory Animal Care, and were in compliance with the guidelines of that association and the EPA/NHEERL Institutional Animal Care and Use Committee. Timed-pregnant Wistar–Han rats were obtained from Charles River Laboratory (Raleigh, NC) on gestation day (GD) 1–5. Dams were assigned to treatment groups by randomization, to ensure equivalent body weight distribution across the groups. Each treatment group consisted of approximately 35 dams (a total of 348 pregnant dams was used). Each of the 10 treatment groups was divided into five daily blocks, to permit work to be done with manageable numbers. All rats within each block were of the same GD. All animals were individually housed in polypropylene boxes containing Alpha-Dri® bedding (Shepherd Specialty Papers, Watertown, TN), and subject to a 12:12 h light:dark cycle under controlled temperature (22 ± 2 °C) and humidity (40–60%). Through GD 5, all animals had unrestricted access to water and LabDiet 5001 Rodent Diet (PMI LabDiet, Richmond, IN).

2.2. Treatment regimen

The exposure model used in this study was selected to accommodate investigation of both developmental neurotoxicity and reproductive toxicity, the latter being reported herein. Starting on GD 6, pregnant dams were given unrestricted access to water and either control feed (5002 Certified Rodent Diet (Purina)), or feed containing myclobutanil (100, 500, or 2000 ppm), propiconazole (100, 500, or 2500 ppm), or triadimefon (100, 500, or 1800 ppm). All feed was prepared by Bayer CropScience (Kansas City, MO) as part of a materials Cooperative Research and Development Agreement between the U.S. EPA and the U.S. Triazole Task Force (a collaboration of manufacturers of triazole fungicides). The conazoles were dissolved in acetone and then mixed into the feed, which was in meal form. Control feed was prepared by mixing acetone with the feed. Acetone was allowed to evaporate fully from the feed prior to use. Food consumption was determined weekly, to calculate consumed conazole dosages. Dams were weighed once per week on the same day. Dams continued on their assigned diet through gestation, parturition, and lactation. They were allowed to deliver naturally, with the day of delivery designated as postnatal day (PND) 0 for the F_1 offspring.

Pups were weighed on PND 0. The size and gender ratio of each litter was noted. Litters were culled to eight pups on PND 8 using blinded random selection, and the pups weaned on PND 22. Female weanlings were housed two per cage and continued until termination on the same diet as their dams had been exposed to. Ear tags were used to identify dams and weanlings.

2.3. Anogenital distance

On PND 0, pup body weights and anogenital distance (AGD, the distance from posterior edge of the genitalia to the anterior edge of the anus) were measured, and footpads tattooed for identification. AGD was measured under a dissecting microscope fitted with a reticle.