SUITABILITY OF PROPICONAZOLE AS A NEW-GENERATION WOOD-PRESERVING FUNGICIDE

By
William R. Goodwine
Janssen Pharmaceutica, 40 Kingsbridge Road, Piscataway, NJ 08854 U.S.A.

SUMMARY

Propiconazole, an anti-fungal triazole compound of Janssen Pharmaceutica, has been extensively tested to determine its potential use as a wood preserving fungicide. Various tests in both private laboratories and official institutes indicates excellent activity against Basidiomycetes (brown and white rot) and Ascomycetes (blue stain-in-service). The active ingredient seems very resistant to weathering (leaching and evaporation). Penetration profiles for propiconazole, following brush application of LOSP formulations, indicates good lateral penetration into wood. The following mean toxic values (kg a.i./m^3) can be supported by the test results: *Coniophora puteana*: 0.27-0.39, *Gloeophyllum trabeum*: 0.37-0.53, *Poria placenta*: <0.22 and *Coriolus versicolor*: 0.25-0.33. The fungicide is stable in treated wood, and can be analyzed by HPLC or GC methods.

Key words: propiconazole, triazole, TBTN, dichlofluanide, decay, blue stain-in-service, LOSP, weathering, penetration.

INTRODUCTION

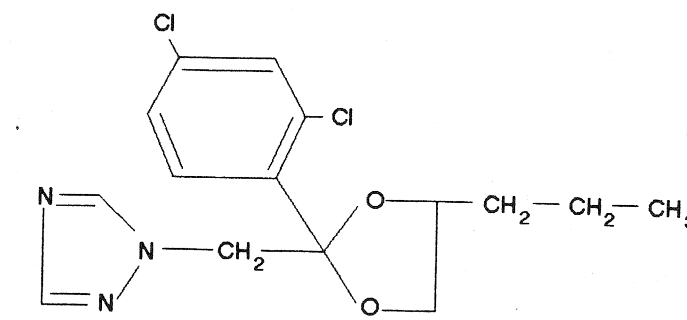
The triazole class of chemicals has been extensively studied by Janssen Pharmaceutica for wood preservation application. Azaconazole has found application primarily in waterborne formulations as a sapstain chemical in different blends. Recently a second triazole commonly known as propiconazole, has been commercialized for wood preservative use. Very promising results have been obtained in light organic solvent preservative (LOSP) systems; water-based formulations are currently under evaluation. Propiconazole has a broad biological spectrum, good solubility characteristics, and has shown good penetration and distribution in the wood. The compound is supported by an extensive data base on toxicology, ecotoxicology, chemistry, and environmental fate.

MATERIALS AND METHODS

Physical-Chemical Properties:

Chemical name: (RS)-1-(2-(2',4'-dichlorophenyl)-4-propyl-1,3 -dioxolan-2-yl-methyl)-1H-1,2,4-triazole

Structural formula:



Molecular formula:	$C_{15}H_{17}Cl_2N_3O_2$
Molecular weight:	342.23
Appearance:	light to dark-yellow, clear highly viscous liquid
Boiling point*:	180 °C (0.13 mbar)
Density:	1.27 g/cm^3 (20 °C)
Vapour pressure*:	1.3×10^{-6} mbar (20 °C) 1.5×10^{-5} mbar (40 °C)
Volatility (SVC)*:	0.019 mg/m^3 (20 °C)
Solubility:	110 ppm in water at room temperature. Very soluble in acetone, methanol, toluene, benzene, octanol, isopropanol, dichloromethaw, propylene glycol. Soluble in hexane, ethylene glycol, mineral spirits.

*determined on pure active substance.

Stability

Thermal:	stable up to ± 350 °C
Chemical:	no significant hydrolysis after 28 days at pH 1 to 13 at a temperature of 70 °C.

Toxicology*

Acute tests:

- oral LD_{50} (rat)	:	1517 mg/kg
- dermal LD_{50} (rat)	:	>4000 mg/kg
- inhalation LC_{50} (rat)	:	>5800 mg/m^3
(4 h/20 °C)		no LC_{50} attainable
- primary eye irritation (rabbit)	:	slight
- primary dermal irritation (rabbit)	:	slight
Skin sensitization (guinea pig)	:	not sensitizing

Aquatic organisms toxicity:

- Daphnia magna LC_{50} (48 h)	:	11.5 mg/l
- Brown trout LC_{50} (96 h)	:	20.0 mg/l

No adverse effects were indicated in sub-chronic, chronic, oncogenicity, teratogenicity, and reproduction studies with propiconazole performed in rats, mice, dogs and rabbits. Several mutagenicity tests (Ames, micronucleus, dominant lethal) demonstrated lack of genetic effects.

*Technical grade material (>85% a.i.) used in all tests.

TEST PROCEDURES

Biological Tests

Modified EN 113 Test

For the preliminary tests carried out in the laboratories of Janssen Pharmaceutica, pine (*Pinus sylvestris L.*) and beech (*Fagus sylvatica L.*) blocks, measuring 5 x 2 x 0.6 cm, were impregnated under vacuum with propiconazole dissolved in a water/ethanol mixture (50/50 w/w) or in xylene. After drying at room temperature for two weeks, the treated samples were placed in Petri-dishes in which the test fungi had grown for two weeks. The beech blocks were exposed to a white rot fungus *Coriolus Versicolor* strain CTB 863 A and the pine blocks to the brown rot fungi *Coniophora puteana* strain BAM 15, *Gloeophyllum trabeum* strain BAM 109, *Poria placenta* strain FPRL 280 and *Serpula lacrymans* strain BFH B 315.

After eight weeks of incubation at 22 °C and 70% R.H., the percentage of weight loss of the wood blocks was calculated and toxic values were determined.

AWPA Standard Soil Block Test (M10-77)

The soil block decay tests were carried out in accordance with the ASTM D 1413 standard. The fungi used were *Gloeophyllum trabeum* strain MAD 617, *Poria placenta* strain MAD 698 and *Coriolus versicolor* strain MAD 697. Sapwood blocks (19 mm cubes) were treated with a series of gradient concentrations of propiconazole for evaluation against all three fungi. Southern yellow pine blocks were used for *Gloeophyllum trabeum* and *Poria placenta*. Sweetgum blocks were used for *Coriolus versicolor*. All the treated blocks were subjected to weathering before exposure to the fungi. The test was carried out at the Mississippi State University, Forest Products Utilization Laboratory.

European Standard EN 113 test

The activity of propiconazole in an organic solvent based formulation against Basidiomycetes was determined according to the European Standard Specification EN 113. This standard decay test consists of exposing small wooden blocks (50 x 25 x 15 mm), treated with various concentrations of preservative, for a fixed period of time (16 weeks at 22 °C and 75% R.H.) to the attack of a vigorously growing pure culture of a wood rotting fungus on agar in Kolle flasks. The blocks are sterilized by gamma-irradiation (1.2 Mrad) to avoid chemical and physical changes of the preservative. Both treated and untreated blocks are exposed at the same time. Vacuum-impregnated Red Pine blocks were exposed to *Coniophora puteana* strain BAM 15 and *Gloeophyllum trabeum* strain BAM 109. Beech blocks were exposed to *Coriolus versicolor* strain CTB 863 A. By noting the weight losses at the end of the test, toxic values of the preservative are determined. These are defined as two values, the lower is that retention of preservative which just allows fungal decay to proceed and the higher represents that retention which just prevents decay(dry weight loss $\leq$ 3%).

NWMA Soil Block Test - Part A

To evaluate the utility of propiconazole for use in the treatment of exterior millwork, a preservative system incorporating the preservative, binder, and water repellent in mineral spirits was developed. Efficacy was determined in the NWMA Soil Block Test Procedure A. Ponderosa Pine sapwood blocks (9mm cubes) were impregnated with an appropriate series of ingredient concentrations of propiconazole ranging from 0.02-0.16%. The percent active ingredient for each treatment level was confirmed using HPLC. The test fungus was *Gloeophyllum trabeum* (MAD 617, ATCC 11539). All the treated blocks were subjected to weathering before exposure to the fungus.

Determination of the decay activity after brush application

A test based on the German Standard procedure RAL-GZ 830 was used. Twelve pine sapwood blocks (5 x 2 x 1.5 cm) for each tested concentration were end-sealed with a 1:1 mixture of ethylacetate and a nitrocellulotic compound. After 3 days drying at 20 °C the wood was oven-dried at 104 °C for 24 hours. Six blocks for each concentration were treated by pipetting 210 ml product/m^2 on the remaining four uncoated sides. Thereafter, the wood was end-sealed with 2 coats of the ethylacetate/nitrocellulose mixture and sterilized by gamma-irradiation (1.2 Mrad). The blocks were then exposed to the fungi. A treated and an untreated block were each time placed together in a culture bottle with the brown-rot fungus *Gloeophyllum trabeum* strain BAM 109 (grown for 14 days on malt agar at 22 °C and 70% R.H.). After 16 weeks exposure at 22 °C and 70% R.H. the wood was oven-dried again and the percentage weight loss was calculated. A concentration is considered efficacious when the mean weight loss of the 6 replicates is $\leq$ 3%.

European Standard EN 152 - part 1 test

The activity of propiconazole, in an organic solvent based formulation, against blue stain-in-service was determined according to the European Standard Specification EN 152 - part 1 - Brushing Procedure.

Pine sapwood blocks (110 x 40 x 10 mm) were treated with formulations containing either 0.6 % or 1.2% propiconazole. The amount of product applied was about 100 ml/m^2. Half of these blocks were coated with an unpigmented varnish without any fungicidal or fungistatic components. The other half were left without a varnish coating.

All samples were then exposed in an Atlas Weatherometer Ci35 for 4 weeks, using the following weathering schedule: Light source: Xenon-lamp, Watts: 3000 Watts, UV: 340 nm (0.35 W/m^2), Filters: intern-quartz and extern-borosilicate, T °C: 45-50, R.H. %: 77-93, Water sprinkling: direct spraying on panels. Program Cycle: 4h light + water spray / 2h light (drying) / 10h light + water spray / 2h light (drying) / 5h light + water spraying / 1h dark (drying). No. of cycles: 28. Total time: 672h.

After this accelerated weathering procedure, the wood blocks were exposed to *Aureobasidium pullulans* strain P 268 and *Sclerophoma pityophila* strain S 231 for 6 weeks. At the end of this test, the amount of surface blueing and the internal blue stain free zone was determined according to the following index:

0 - No blue stain
1 - Insignificant stain: only individual small spots
2 - Blue stained: $\leq$ 1/3 continuously stained
3 - Severe stain: > 1/3 surface continuously stained.

A product containing the same inerts and 0.6% dichlofluanide was included as reference preservative.

Weathering Procedures

Leaching Test

Leaching tests were carried out according to the European Standard Leaching Procedure EN 84. Wood blocks were vacuum-impregnated at 40 mbar with distilled water for 20 min. The vacuum was then broken, and the samples were left soaking for 2 hours. The samples were then submerged for 14 days, with 9 water volume changes during this period. The blocks were removed from the water and air-dried. The leaching water from these tests was pooled and analyzed to detect the amount of propiconazole leached from the wood. The leaching percentage was calculated on the basis of the uptake.

Evaporative ageing

Evaporative ageing tests were carried out by exposing the treated samples to 40 °C for 4 weeks or to 80 °C for 2 weeks. Accelerated ageing was also done following the European Standard EN 73 Procedure. Vacuum-impregnated wood blocks were exposed in a wind channel at 40 °C for 12 weeks, in an air current with a mean velocity of 1 m/s.

Stability research

Stability of propiconazole in wood

Vacuum-impregnated blocks were analyzed: immediately after treatment, after 4 weeks conditioning, after a leaching procedure according to EN 84 and after evaporative ageing at 50 °C for 4 weeks.

Study of the penetration profile

Pine sapwood blocks (50 x 50 x 20 mm) were end-sealed and treated with different organic solventborne formulations, containing 6 g/l, 12 g/l or 24 g/l propiconazole. Five blocks were treated per concentration. The products were brush-applied at either 140 ml/m^2 or 220 ml/m^2. For each block the amount of propiconazole was determined by GC-analysis in 1 mm increments up to a depth of 8 mm. The influence of the addition of a hydrocarbon binder on the penetration profile was checked as well. Two formulations (12 g/l and 24 g/l Propiconazole) were applied at a mean rate of 120 ml/m^2 with and without 10% hydrocarbon resin.

RESULTS AND DISCUSSION

Biological Results

As can be seen from Table 1, the toxic values reveal good activity against both brown and white rot fungi. Weathering (leaching) had no negative influence on the biological performance of propiconazole. It is interesting to note that propiconazole is also active against the dry rot fungus *Serpula lacrymans.*

Table 1: Toxic values of propiconazole in a modified EN 113 test.

Formulation: propiconazole technical
Exposure duration: 8 weeks

Toxic Values (kg a.i./m^3)

Fungus	Unaged	Leached (EN 84)	Solvent
C. puteana BAM 15	0.19-0.37	N.T.	50/50 % w/w EtOH/H_2O
P. placenta FPRL 280	0.12-0.24	N.T.	
G. trabeum BAM 109	0.19-0.38	N.T.	
C. versicolor CTB 863 A	0.16-0.33	N.T.	
S. lacrymans BFH B 315	0.17-0.58	N.T.	
C. puteana BAM 15	0.15-0.28	0.15-0.28	xylene
G. trabeum BAM 109	0.29-0.55	0.14-0.28	
C. versicolor CTB 863 A	0.19-0.41	0.19-0.40	

N.T. = Not Tested

The toxic values presented in Table 2, obtained in the AWPA soil-block test against both *Gloeophyllum trabeum* strain MAD 617 and *Poria placenta* strain MAD 698 corroborate the toxic limits found in the European standard tests. The use of a soil medium instead of agar, as well as the fact that other fungal strains and softwood species were used, had no influence on the performance of the compound.

The threshold retention towards *Coriolus versicolor* strain MAD 697 on sweetgum was 0.046 PCF. The sweetgum controls showed an average weight loss of 65.8 %, which is twice as high as average weight losses obtained with beech blocks.

Table 2: Threshold retention of propiconazole in an AWPA soil-block test (weathered blocks).

Formulation: propiconazole technical in acetone
Exposure duration: 12 weeks

Fungus	Threshold Retention (kg a.i./m^3)	Concentration (% a.i.)	Average Weight Loss (%)		Wood Species
			Treated Blocks	Control Blocks	
G. trabeum MAD 617	0.22 0.48	0.05 0.10	24.4 1.8	43.8	SYP
P. placenta MAD 698	0.22	0.05	0.06	37.6	SYP
C. versicolor MAD 697	0.66 0.74	0.16 0.21	29.3 2.5	65.8	Sweetgum

A solventborne formulation containing 1.2% propiconazole, 0.4% permethrine (25:75 cis:trans), 10% hydrocarbon resin and Shellsol H as solvent was tested. From Table 3 it can be seen that propiconazole had excellent activity against both brown rot fungi *Coniophora puteana* strain BAM 15, and *Gloeophyllum trabeum* strain BAM 109 as well as towards the white rot fungus *Coriolus versicolor* strain CTB 863 A. Leaching and evaporative ageing did not significantly reduce the decay activity of propiconazole. Only with *Gloeophyllum trabeum* strain BAM 109 after 12 weeks evaporative ageing, was a slight increase of the toxic limits noted.

Table 3: Toxic values of propiconazole determined in an EN 113 test, after leaching (EN 84) and after evaporative ageing (EN 73).

Formulation: 1.2% propiconazole, 0.4% permethrine, 10% hydrocarbon resin in Shellsol H
Exposure duration: 16 weeks

Toxic Values (kg a.i./m^3)

Fungus	Unaged (EN113)	Leached (EN113+EN84)	Evaporative aged (EN113+EN73)
C. puteana BAM 15	0.27-0.38	0.19-0.30	0.31-0.39
G. trabeum BAM 109	0.39-0.50	0.37-0.49	0.57-0.64
C. versicolor CTB 863 A	0.25-0.33	0.16-0.20	0.33-0.42

The weight loss and retention data from the NWMA soil block test are presented in Figure 1 and Figure 2. If the range of retentions has been appropriate, two groups of points will be apparent, both so distributed as to define intersecting straight lines. These lines represent those losses due to decay that are correlated with retention and operational weight losses. The point of intersection defines the threshold retention of 0.487 kg/m^3. This retention corresponds to solutions of active ingredient concentrations of 0.09-0.1%.

Figure 1: Propiconazole retention and block weight loss

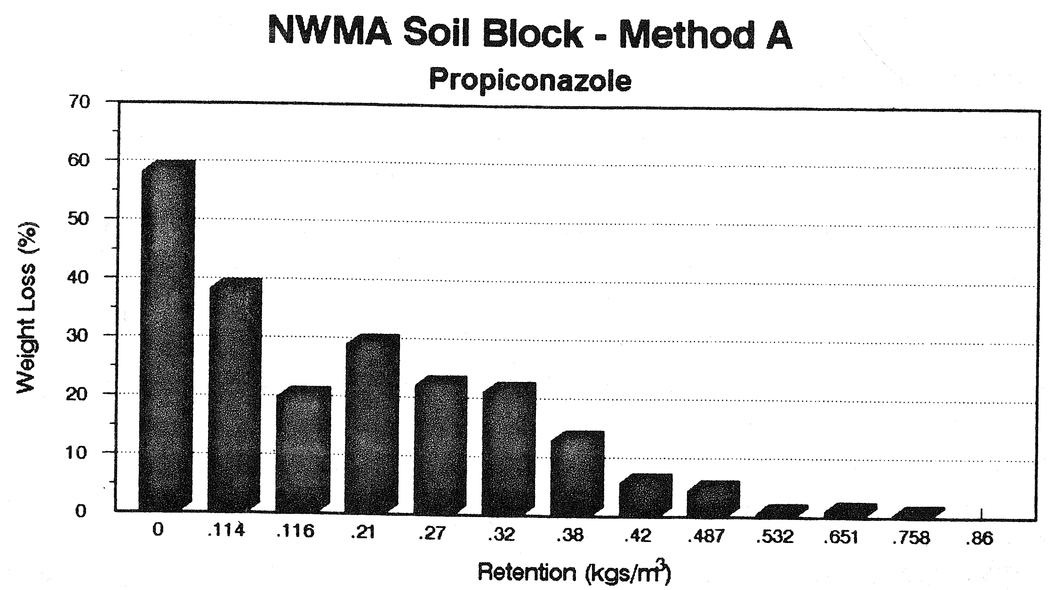
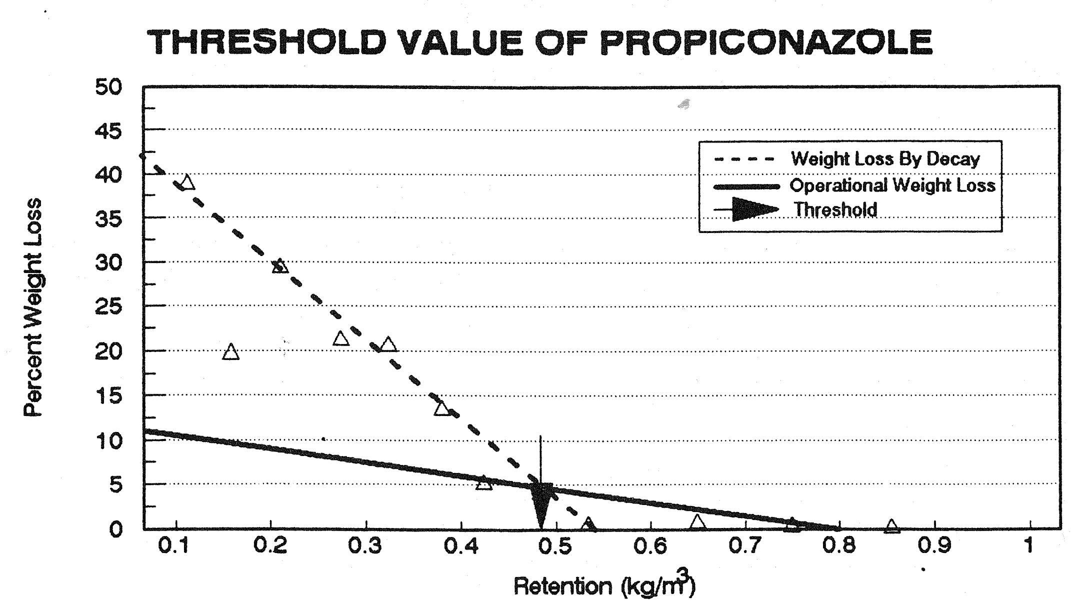


Figure 2: Propiconazole threshold value



Testing of leached versus unleached blocks did not exhibit any difference in the weight loss of the blocks (Table 4).

Table 4: Comparison of loss of weight between unleached and leached treated blocks

Level of Active Ingredient in the Treatment	Percent Weight Loss in Unleached Blocks	Percent Weight Loss in Leached Blocks
0.12	0.00 ± 0.0	0.91 ± .97
0.14	0.57 ± .68	0.42 ± .19
0.16	0.47 ± .53	0.0 ± 0.0

In order to detect the decay activity of propiconazole applied by superficial (brush) treatments, some preliminary tests were carried out in the Janssen laboratories. *Gloeophyllum trabeum* strain BAM 109 was used as the test fungus since this strain required the highest toxic threshold (Table 3). From the data in Table 5 it can be concluded that 1.2% propiconazole, offers good decay control by brush application. It should also be stressed that the formulation type, especially the binders, have a great influence on the final results. This is mainly due to their interference in the penetration and distribution of the active ingredients in the wood tissues.

Table 5: Determination of the decay activity from propiconazole when applied by brush.

Exposure duration: 16 weeks
Retention: 210 ml/m^2
Fungus: *Gloeophyllum trabeum* BAM 109 Average Weight Loss (%)

Formulation	% a.i. Propiconazole	Treated Blocks	Control Blocks
Shellsol H 10% hydrocarbon resin	1.2	+ 9.6	48.0
Shellsol H 10% copolymer resin	1.2	2.8	40.0
Shellsol H	1.0	3.9	34.0
Shellsol H	2.0	0.0	28.0

The effectiveness of propiconazole for preventing blue stain-in-service was compared to a commercial standard treatment of 0.6% dichlofluanide. The results from Table 6 indicate that 0.6% propiconazole gives the same degree of protection against blue stain-in-service as the reference fungicide. It should be emphasized that a very simple formulation, (without binders as normally used in exterior wood stains) was tested. This probably also explains the rather high surface blueing score for dichlofluanide.

Table 6: Assessment of the effectiveness of propiconazole for preventing blue stain-in-service according to EN 152 - part 1.

	Chemical					
Coating	0.6% Dichlofluanide		0.6% Propiconazole		1.2% Propiconazole	
	(1)	(2)	(1)	(2)	(1)	(2)
–	1.9	7.3	1.6	6.1	1.5	5.4
+	1.1	7.8	1.1	6.7	1.1	8.3

(1) Surface assessment (2) Mean stain free zone (mm)

Stability of Propiconazole

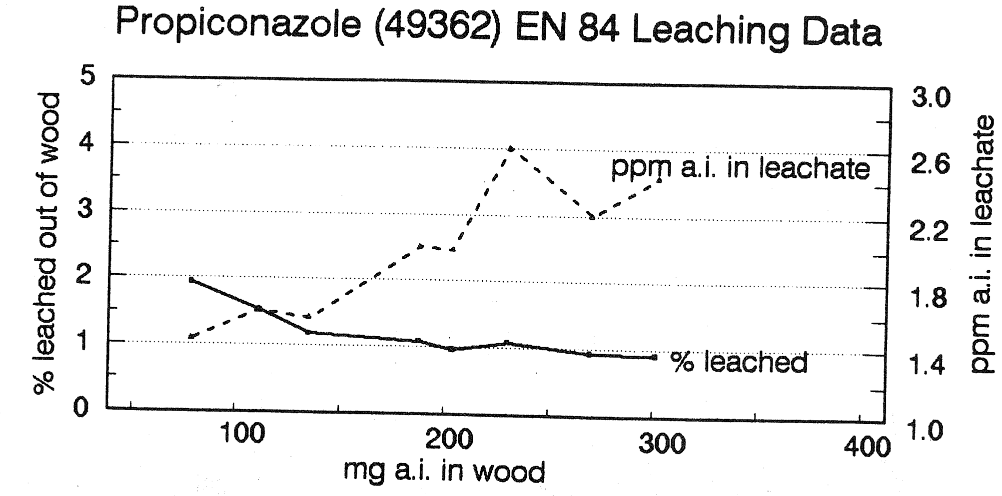
The tests used to assess the biological performance of propiconazole under different weathering stresses are an indication of the compound's stability in treated wood. Analysis of 14C-labelled propiconazole confirmed the chemical stability in treated wood; there was no depletion of the chemical from the wood following various weathering stresses (Table 7).

Table 7: Vacuum-impregnated wood with 14C-labelled propiconazole in xylene

Exposure Condition	Propiconazole Concentration (mg/g wood)		Number of Blocks
	Mean	STD	
After treatment	6.24 ±	0.22	18
After 4 weeks conditioning	6.44 ±	0.30	18
After leaching stress (EN 84)	6.69 ±	0.09	6
After volatilization stress (4 weeks - 50°C)	7.11 ±	0.47	6

The leaching waters were pooled for each tested concentration and analyzed by using a GC-method at Janssen Pharmaceutica. It was found that the percentage leaching at each tested retention was lower than 2% (Figure 3). In other words, at least 98% of propiconazole remained in the wood. These results explain the excellent biological performance after leaching. Moreover, the active ingredient concentrations in the leaching water, are well below the established LC50 values for aquatic organisms. Therefore, it can be anticipated that the professional use of propiconazole will not result in significant environmental pollution.

Figure 3: Leaching of propiconazole from wood determined according to EN 84.



Penetration of Propiconazole into Wood

The study of the mechanism of penetration and distribution of propiconazole into wood was carried out using LOSP formulations containing 6, 12 or 24 g/l a.i., applied by brush at the rate of 140 or 220 ml/m². The penetration profiles given in Figures 4 and 5 indicates the good lateral penetration of propiconazole into wood. This corroborates the mean stain free zones detected with the EN 152 test (Table 6), and the good decay activity of propiconazole when applied by brush (Table 5). When used at 2% w/w at a rate of 220 ml/m² propiconazole is able to protect the wood up to a depth of 8 mm, by a simple brush application. These results also indicate that an effective envelope treatment can be expected when propiconazole will be applied by double-vacuum treatments. Since many LOSP's are formulated with binders, the influence of a 10 % hydrocarbon resin on the penetration profiles of propiconazole into the wood was also investigated. As can be seen from Figure 6, the addition of the hydrocarbon resin used did not change the penetrability of the active ingredient.

Figure 4: Penetration profiles of propiconazole brush-applied at 140 ml/m² at different concentrations.

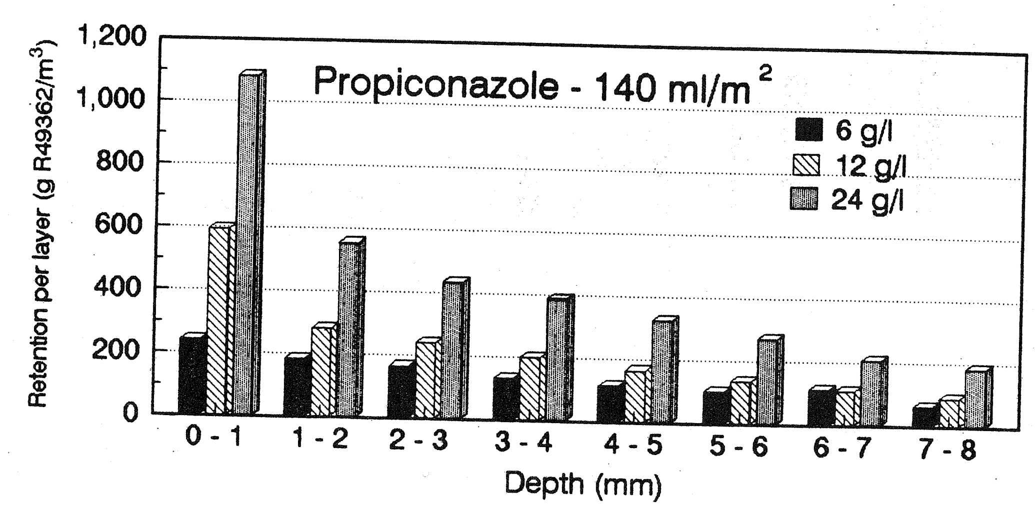


Figure 5: Penetration profiles of propiconazole brush-applied at 220 ml/m^2 at different concentrations.

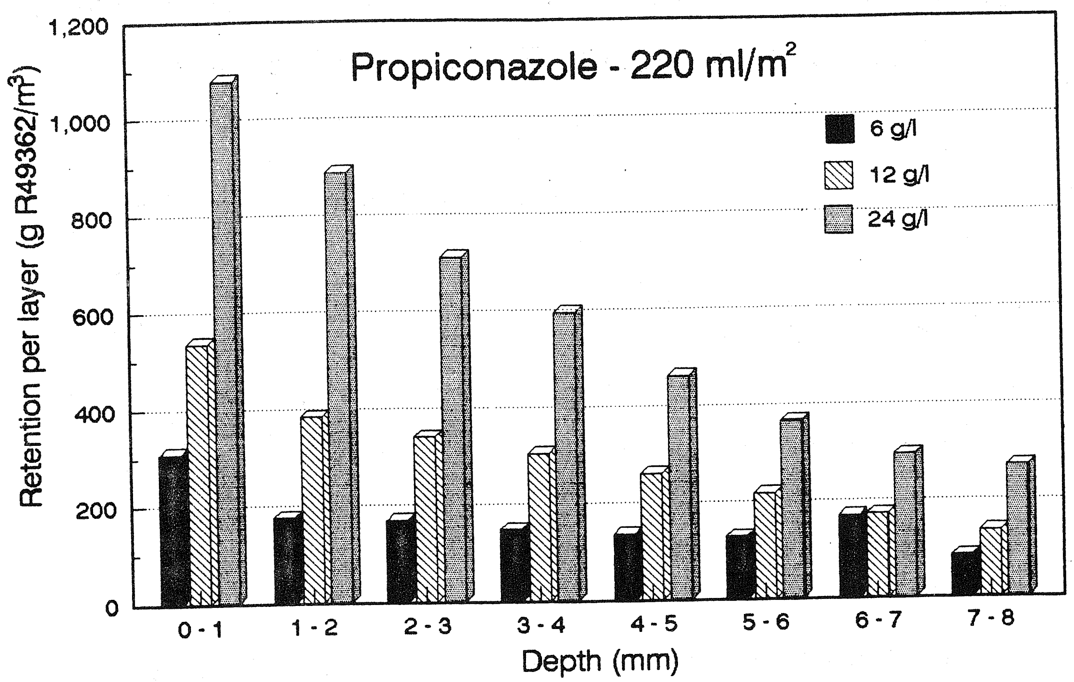
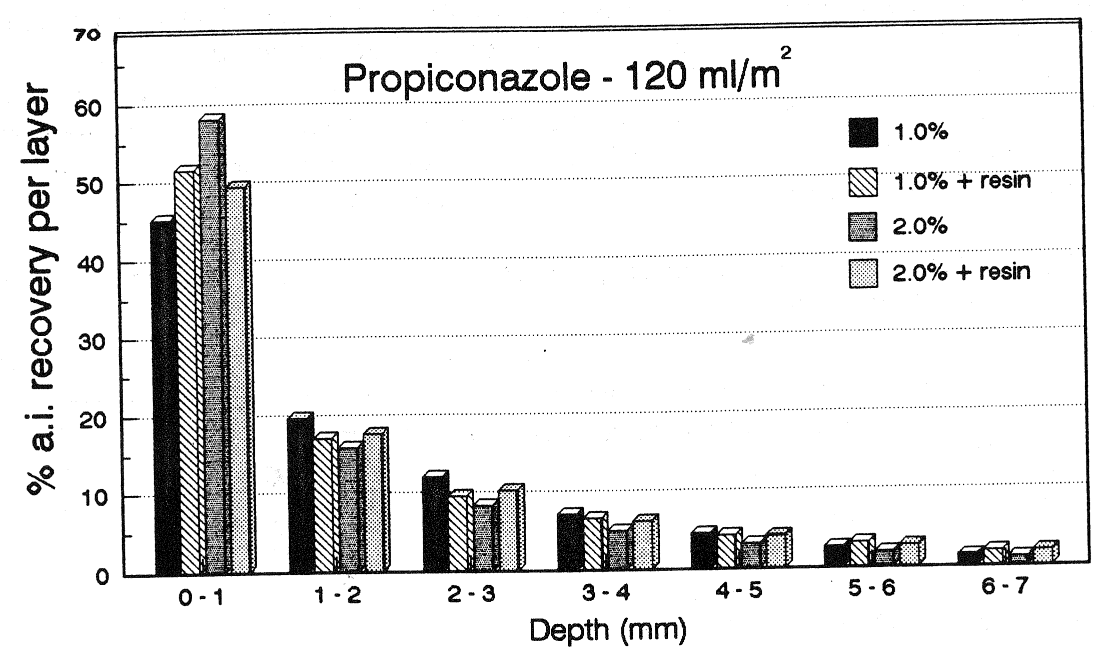


Figure 6: Penetration profiles of propiconazole applied at 120 ml/m^2 at 2 different concentrations with and without hydrocarbon binder.



CONCLUSION

Propiconazole shows considerable promise for wood preservation application based on the biological results presented. Propiconazole exhibits excellent activity against wood destroying Basidiomycetes (brown rot and white rot, including the dry rot fungus *Serpula lacrymans*). The active ingredient is resistant to both leaching and evaporative ageing. Propiconazole is also suited for preventing blue stain-in-service. Propiconazole remains stable in treated wood. Retention of propiconazole in wood following brush application provides decay and blue stain control to a depth of 8 mm.

REFERENCES

AWPA (1989) - Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures (M10-77).

CEN (1978) - EN 73 Wood Preservatives. Accelerated ageing of treated wood prior to biological testing.
Evaporative ageing procedure.
European Committee for Standardization, First edition.

CEN (1979) - EN 84 Wood Preservatives. Accelerated ageing of treated wood prior to biological testing.
Leaching procedure.
European Committee for Standardization, First edition.

CEN (1980) - EN 113 Determination of toxic values of wood preservatives against wood destroying basidiomycetes cultured on an agar medium.
European Committee for Standardization, First edition.

CEN (1984) - pr EN 152 Draft 2 - Wood Preservatives. Laboratory method for determining the protective effectiveness of a preservative treatment against blue stain-in-service - part 1 - brushing procedure.

RAL (1987) - Holzschutzmittel Gutesicherung
RAL-GZ 830 - Ausgabe September 1987.
Deutsches Institute fur Gutesicherung and Kennzeichnung.