## FENBUCONAZOLE (197)

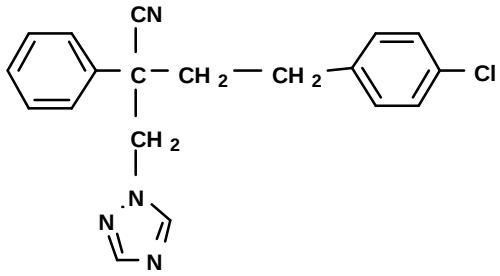
ISO common name: fenbuconazole

Chemical name
IUPAC: 4-(4-chlorophenyl)-2-phenyl-2-(1*H*-1,2,4-triazol-1-yl)methybutyronitrile
CA: α-[2-(4-chlorophenyl)ethyl]-α-phenyl-1*H*-1,2,4-triazole-1-propanenitrile

CAS registry no: 114369-43-6 (unstated steriochemistry)
119611-00-6 (with chiral properties specified)

Synonyms: Fenethanil, RH-7592, RH-57592.

Structural formula:




Molecular formula: $C_{19}H_{17}ClN_4$

Molecular weight: 336.8

**Physical and chemical properties**

Pure active ingredient

Physical state: solid

Appearance: white crystalline

Melting point: 126.5-127.0°C (Graves, 1991; Batra 1997)

Solubility: water, mg/l: 3.77/25°C; 7.01/40°C (bead elution/HPLC method, unbuffered water) 2.04-2.26 (22°C shake flask method, unbuffered water) (Jacobson, 1988)

3.6/pH 7; 3.6/pH9 (20°C, shake flask method) (Kellner, 1992) soluble in ketones, esters, alcohols and aromatic hydrocarbons; insoluble in aliphatic hydrocarbons

Vapour pressure: $<10^{-5}$ Pa at 20°C (OECD method 104) (Kellner, 1992)

4.9 x $10^{-6}$ Pa. (Purity unstated but test carried out on "recrystallised fenbuconazole") (Graves, 1991)

Hydrolysis:

Not hydrolysed 30 days under sterile conditions in the dark at pH 5, 7, and 9. Estimated minimum half-life 2210 days at pH 5, 3740 days at pH 7, 1340 days at pH 9 (O'Dowd, 1990d)

Photolysis:

Stable in pH 7 aqueous buffer at 25ºC for 30 days with 12-hour light/dark cycles (Wang,1991b)

Degraded at a concentration of 1.5mg/l in natural pond water. Assuming pseudo-first-order kinetics, calculated half-life was 86.7 days. (Baur 1994)

Octanol/water partition coefficient:

log $P_{ow}$ = 3.22 at 25ºC. Determined from 14C-fenbuconazole (radiochemical purity 99.5%) solutions of 100, 500 and 1,000 ppm which were partitioned in triplicate between octanol and water, and the concentration in each phase was determined by radioassay. (O'Dowd, 1987).

Henry's Law Constant: 7.63 x $10^{-9}$ [atm.$m^3$/g.mol] at 25ºC (Chong, 1992)

Technical material

Purity: 98.3% (94% minimum)
Physical state: Off-white to white powder
Melting point: 124°-126°C

Solubility in water: 3.8 mg/l at 25°C, 7.0 mg/l at 40°C (unspecified pH) (Graves, 1991; Costlow, 1997b)

Solubility, g/l 25°C: (by HPLC)
231 in acetonitrile
77 in aromatic 200 (C9 & C10 hydrocarbons)
445 in cyclohexanone
159 in ethyl acetate
39 in ethanol
1 in heptane
13 in n-octanol
(Graves, 1991)

Fat solubility at 37°C: 1052.2 mg/100g (MacDonald *et al.*, 1990a; Batra, 1997)

Surface tension:

69.5 mN/m (as a 90% saturated water solution at 22°C. (fenbuconazole purity stated as "94 to 99.5%"). Test carried out according to EU method A5. (MacDonald *et al.*, 1990c; Costlow, 1997b)

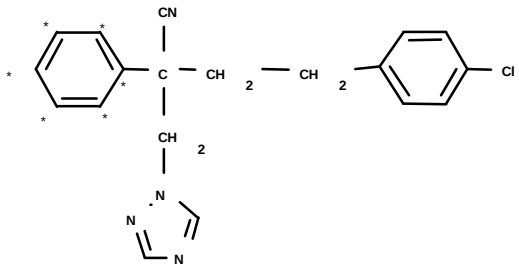
Relative density: $D^{20}$ = 1.27 (EU method A3) (MacDonald *et al.*, 1990b)

## Formulations

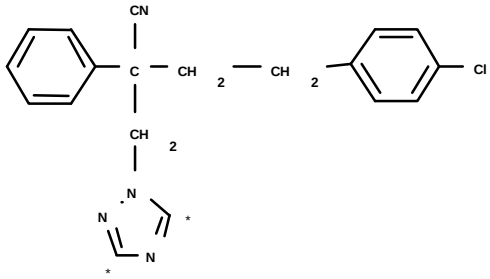
Fenbuconazole is a triazole fungicide and is formulated mainly as an EC or an EW (oil in water emulsion).

## METABOLISM AND ENVIRONMENTAL FATE

The studies were carried out with [$^{14}$C]fenbuconazole uniformly incorporated in the unsubstituted phenyl ring (phenyl-labelled) or the triazole ring ("triazole-labelled"), as shown by the asterisks in the structures shown below.




Phenyl-labelled




Triazole-labelled

### Animal metabolism

Rats. In a 1987 study (Hanauer, 1991), four groups of 4 Crl:CD BR rats were orally dosed with phenyl-labelled [$^{14}$C]fenbuconazole (radiochemical purity >99%). A 0.5% suspension of methyl cellulose was used as the vehicle. Dosing was by gavage at 100 mg/kg bw. All animals were killed 7 days after dosing.

In group 1 (four males) whole blood samples were taken for liquid scintillation counting 0.25, 1, 3, 6, 24, 48, 72, 96 and 168 hours after dosing. In group 2 (four males) urine and faeces were collected at 0, 6, 24, 48, 72, 96 and 168 hours, and $^{14}$C was determined in the expired air and in selected tissues and organs after 7 days. In groups 3 and 4 (four animals of each sex) urine and faeces collected as in group 2 were frozen over liquid nitrogen for subsequent analysis.

The average total recovery of $^{14}$C from the animals in group 2 was 67% of the administered dose, about 62% in the faeces and 4% in the urine. Radioactivity detected in expired $CO_2$ accounted for only 0.05% of the administered dose. Most of the excretion had occurred by 48 hours. Peak blood and plasma levels of the radiolabel were detected at 6 hours. A biphasic elimination pattern was seen with a rapid first phase (half-life 7 hours) followed by a slower second phase (half-life about 50 hours for plasma and 187 hours for whole blood). After 7 days <0.5% of the administered dose was detected in the tissues, where the radiolabel levels were all lower than 2.5 mg/kg fenbuconazole equivalents (liver levels were highest at 2.48 mg/kg or 0.13% of the dose). Of the average total of 0.53% of the dosed radiolabel found in the bodies after 7 days, 0.24% was found in the carcase.

The combined faeces and urine samples of each sex were analysed, using a variety of extractions, solvent partitions and chromatographic separations, and the purified components of the residue were identified by mass spectrometry or chromatographic comparison with synthesized samples of the suspected metabolites. The metabolite profile in the excreta is shown in Table 1.

Table 1. Metabolite profile of fenbuconazole in excreta of rats.

| Compound | Percentage of total activity in excreta | |
|---|---|---|
| | Male | Female |
| Fenbuconazole | 7.9 | 13.0 |
| Lactones (both diasterioisomers) | 12.9 | 8.1 |
| Iminolactone | 2.5 | 0.9 |
| Benzylic alcohols (including non-sulfate conjugates) | 7.1 | 3.3 |
| Benzylic sulfates (both diasterioisomers) | 4.1 | 11.2 |
| Phenols (3-OH and 4-OH isomers, including conjugates) | 9.3 | 14.3 |
| Phenol lactones (both isomers, including conjugates) | 6.5 | 5.8 |
| Ketoacid | 2.4 | 1.0 |
| Phenol ketoacid | 5.1 | 0.0 |
| RH-7968[1] | <1 | <1 |
| Triazole | 1.9[2] | 1.5[2] |

[1]4-(4-chlorophenyl)-2-hydroxymethyl-2-phenylbutyronitrile
[2]Percentage of dose

In a 1992 study, groups of male and female Crl:CD BR rats were dosed by gavage with phenyl-labelled [$^{14}$C]fenbuconazole (98.7% radiochemical purity) using a 0.5% suspension of methyl cellulose as the vehicle. Three groups of 5 males and 5 females were given single doses of 1 or 100 mg/kg bw [$^{14}$C]fenbuconazole or a labelled dose of 1 mg/kg bw after a 14-day treatment with 10 ppm of the unlabelled compound in the diet. Urine and faeces samples were taken at 0, 6, 24, 48, 72 and 96 hours after dosing and the animals were then killed. Two groups of 3 males and 3 females were dosed with 1 mg/kg bw, and excreta samples were taken at the time of dosing and when the rats were killed after 3 or 12 hours. One group of 5 males and 5 females were biliary canulated and dosed at 1 mg/kg bw. Excreta samples were taken up to 72 hours, when the animals were killed, and blood samples at 0, 6, 24, 48 and 72 hours. Tissues, organs and the carcases except those from the last group were analysed for radioactivity, but only the whole carcases of the canulated group were analysed.

The average total recoveries of $^{14}$C in the groups killed after 4 days were 96-104.7% of the administered doses, mainly in the faeces and within the first 48 hours (Table 2).

Table 2. Recovery of [$^{14}$C] from tissues and excreta of rats 4 days after gavage administration of [$^{14}$C]fenbuconazole.

| Dose, mg/kg bw | Sex | $^{14}$C, mean % of administered dose | | | | |
|---|---|---|---|---|---|---|
| | | Faeces | Urine | Tissues | Carcase | Total[1] |
| 1 | Male | 85.3 | 9.8 | 0.5 | 0.3 | 96.0 |
| 1 | Female | 92.2 | 11.4 | 0.8 | 0.1 | 104.7 |
| 100 | Male | 90.2 | 7.0 | 0.2 | 0.2 | 97.6 |
| 100 | Female | 78.7 | 11.1 | 0.2 | 0.2 | 90.4 |
| 1[2] | Male | 94.4 | 8.7 | 0.6 | 0.3 | 104.3 |
| 1[2] | Female | 89.4 | 11.1 | 0.8 | 2.9 | 104.5 |

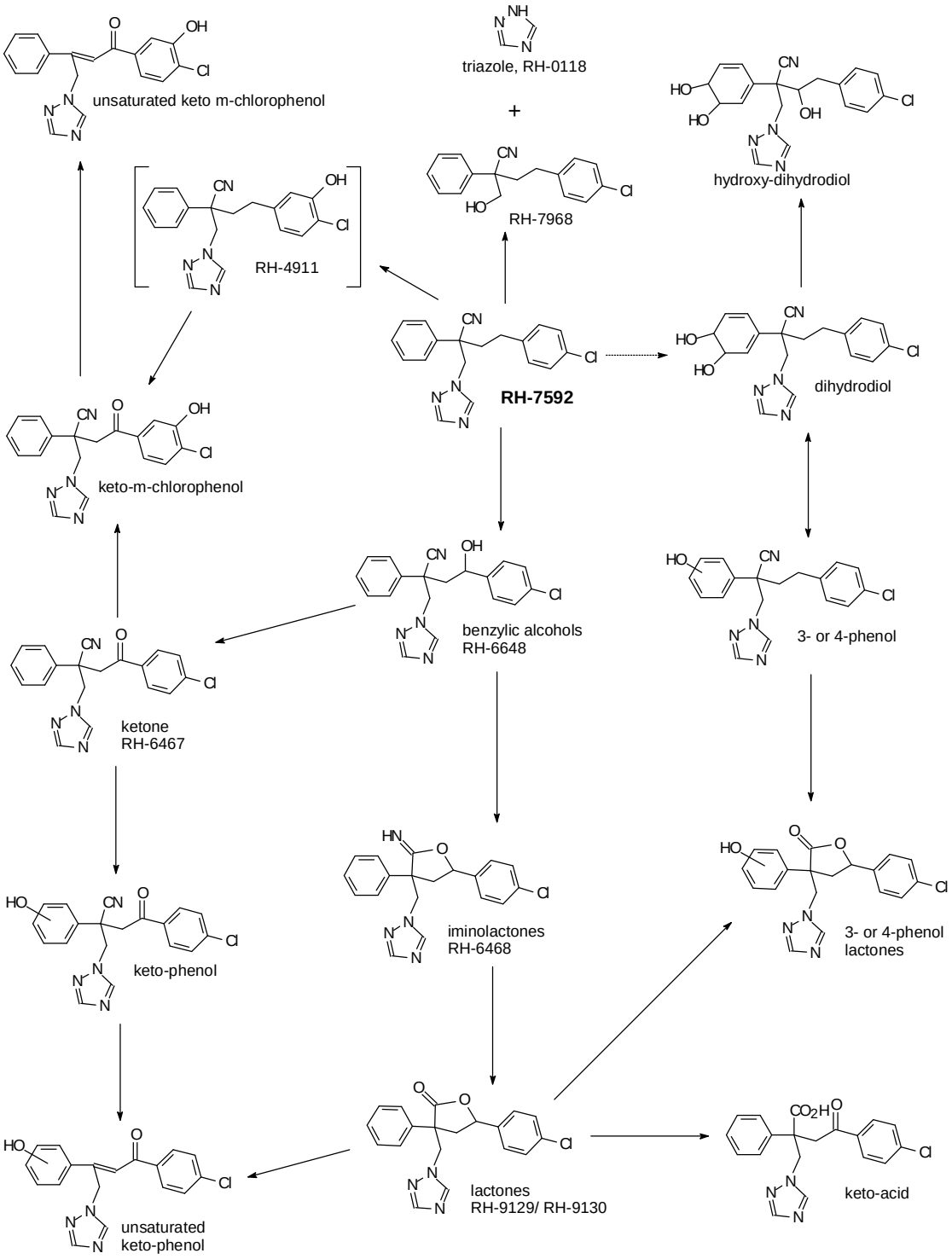
[1]Including cage wash
[2]After treatment for 14 days with 10 ppm unlabelled fenbuconazole in the diet

The results did not show any significant sex- or dose-related differences between treatments. In the canulated animals during the 3 day collection period, averages of 79-87% of the total dosed radiolabel were found in the bile (mainly within 24 hours), 6-7% in the faeces, 3-7% in the urine and 1-2% in the carcase, with total recoveries of 95-98%; 88-91% of the doses were absorbed.

The average total $^{14}$C in the tissues of animals killed 3 and 12 hours after dosing was 8-12% and 3-4% of the administered dose respectively, of which >90% was found in the liver. The total residues of radiolabel in the carcases 4 days after dosing were low. At 1 mg/kg bw (both with and without prior dietary administration) the average residues varied from 0.001 mg/kg fenbuconazole equivalents in the muscles to maxima of 0.13 mg/kg in the liver (females) and 0.19 mg/kg in the thyroid (males). At 100 mg/kg bw $^{14}$C levels varied from 0.09 mg/kg in the muscles to 0.7-1.4 mg/kg in the thyroids of males and 3.1-4.2 mg/kg in the livers of females (Donato and Hazelton, 1993).

On the basis of these studies the manufacturer proposed the metabolic pathways for fenbuconazole in rats shown in Figure 1.

Figure 1. Metabolism of fenbuconazole in rats.




The hydroxylated compounds may also be conjugated with sulfate, but were mainly present as glucuronide (Batra, 1997; Ross, 1997b).

Goats. Nine lactating goats each received seven daily doses of [$^{14}$C] fenbuconazole, five with the phenyl label at rates equivalent to 1, 10 and 100 ppm in the feed and four with the triazole label at a rate equivalent to 100 ppm. The goats were slaughtered 24 after the last dose. All samples were analysed by LSC after combustion. The samples were also extracted with

methanol and then partitioned successively with ethyl acetate, n-butanol, hexane and water. The resulting organic and aqueous phases were analysed by TLC and HPLC.

The overall recoveries of $^{14}C$ were 72.7-87.3% from the phenyl label and 74.3-81.2% from the triazole labels. Most of the radioactivity was excreted (79.7-86.0% of the phenyl label and 72.3-80.0% of the triazole), with less than 0.1% of the phenyl label and 0.2-0.4% of the triazole label in the milk and 0.8-1.2% of the phenyl and 1.1-1.6% of the triazole label in the tissues.

The total $^{14}C$ in the milk, expressed as fenbuconazole equivalent, was less than 0.01 mg/kg at the 1 and 10 ppm feeding levels. At the 100 ppm level it reached plateaux of 0.07 mg/kg after 4 days with the phenyl label and 0.4 mg/kg after 5 days with the triazole label.

The triazole-labelled compound yielded two major metabolites in the milk, triazole (0.24 mg/kg) and triazolylalanine (0.15 mg/kg). The parent compound and six other metabolites were also identified, but were present at levels below 0.02 mg/kg (Table 3).

Table 3. Fenbuconazole and its metabolites in goat milk.

| Compound | $^{14}C$, mg/kg as fenbuconazole | |
|---|---|---|
| | Phenyl label | Triazole label |
| Fenbuconazole | 0.02 | <0.01 |
| Lactones and phenols | 0.02 | <0.01 |
| Triazole | - | 0.24 |
| Triazolylalanine | - | 0.15 |
| RH-7968 | <0.01 | <0.01 |
| Benzylic glucuronides | 0.02 | <0.01 |
| Total | 0.06 | 0.40 |

The total $^{14}C$ in the tissues (expressed as fenbuconazole) from the phenyl label was less than 0.05 mg/kg from the 1 and 10 ppm feeding levels, except in liver which contained 0.10 and 0.62 mg/kg. The 100 ppm feeding level produced 0.07 mg/kg in the muscle, 0.16 mg/kg in the fat, 0.89 mg/kg in the kidneys and 7.9 mg/kg in the liver. The triazole label at 100 ppm gave average total $^{14}C$ residues in the tissues of 0.23 mg/kg in the muscle, 0.02 mg/kg in the fat, 0.94 mg/kg in the kidneys and 12.1 mg/kg in the liver.

Table 4 shows the averages of the residues from the two labels for the 100 ppm feeding level.

Table 4. Fenbuconazole and its metabolites in goat tissues.

| Compound | $^{14}C$, mg/kg as fenbuconazole | | | |
|---|---|---|---|---|
| | Liver | Kidneys | Muscle | Fat |
| Fenbuconazole | 0.95 | 0.10 | 0.02 | 0.02 |
| Lactones[1] | 0.84 | 0.06 | <0.01 | <0.01 |
| Phenol | 0.47 | 0.13 | <0.01 | <0.01 |
| Iminolactones[1] | 0.56 | <0.01 | <0.01 | <0.01 |
| Ketoacid | 0.16 | <0.01 | <0.01 | <0.01 |
| Benzylic alcohols[1] | 0.13 | 0.05 | <0.01 | <0.01 |
| Benzylic sulfates[1] | 0.40 | 0.03 | <0.01 | <0.01 |
| Triazole | 1.79 | 0.11 | 0.09 | <0.01 |
| Triazolylalanine | 4.95 | 0.24 | 0.07 | <0.01 |
| RH-7968 | 0.95 | 0.13 | 0.01 | 0.04 |
| Benzylic glucuronides[1] | 1.23 | 0.13 | <0.01 | 0.01 |
| Total | 12.43 | 0.97 | 0.22 | 0.11 |

[1]Diasterioisomers

The six major components identified in the liver were the parent (0.95 mg/kg), the lactones (0.84 mg/kg), 4-(4-chlorophenyl)-2-hydroxymethyl-2-phenylbutyronitrile (RH-7968, 0.95 mg/kg), the benzylic glucuronides (1.23 mg/kg), triazole (1.79 mg/kg) and triazolylalanine (4.95 mg/kg). Five other metabolites were identified at levels below 0.6 mg/kg.

In the kidneys the six main components were the parent (0.10 mg/kg), RH-7968 (0.13 mg/kg), the phenol (0.13 mg/kg), glucuronides (0.13 mg/kg), triazole (0.11 mg/kg) and triazolylalanine (0.24 mg/kg). Five other metabolites were identified at levels of [0.06 mg/kg.

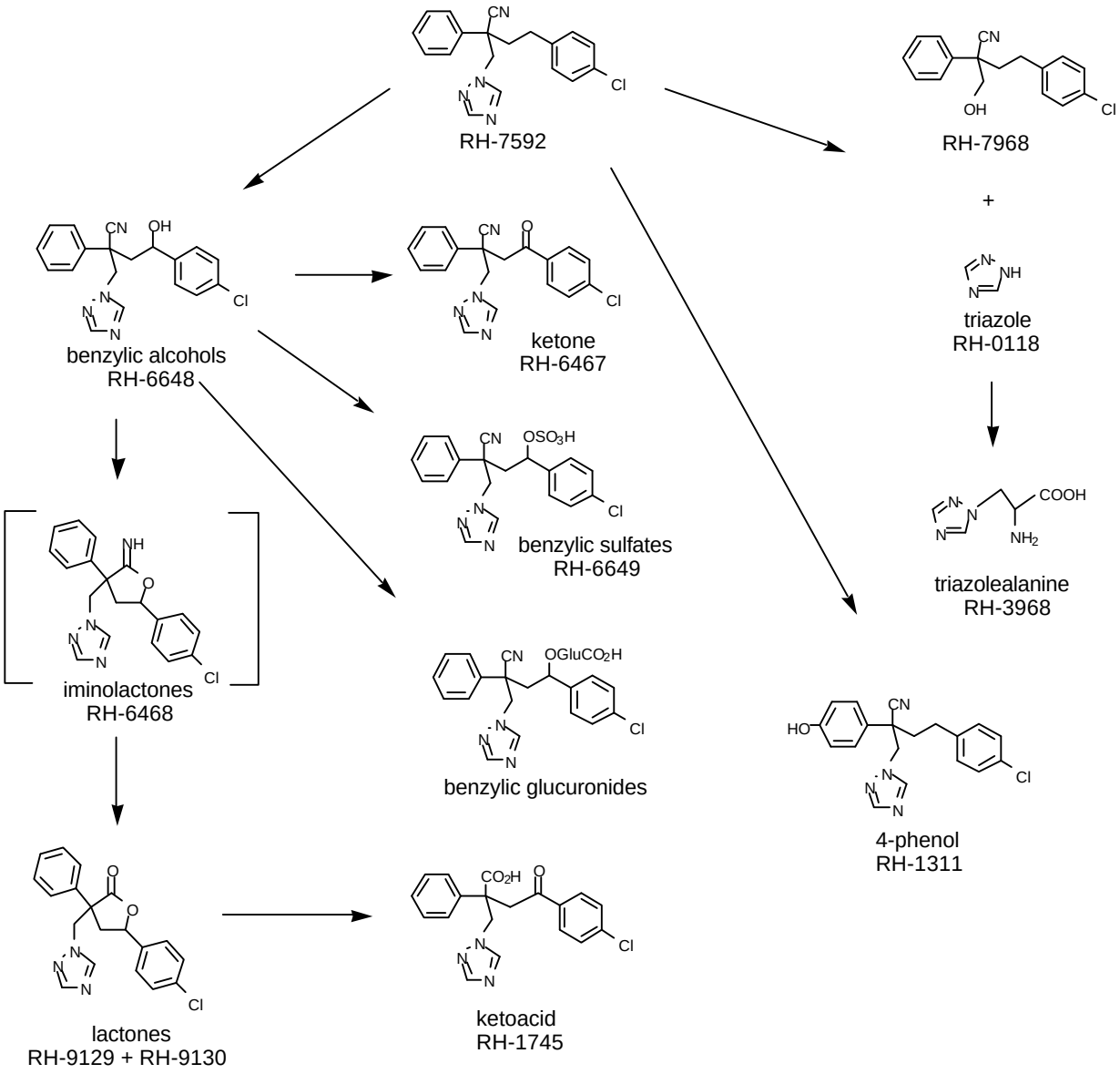
In muscle the main components were the parent (0.02 mg/kg), RH-7968 (0.01 mg/kg), triazole (0.09 mg/kg) and triazolylalanine (0.07 mg/kg). Six other metabolites were identified at levels below 0.01 mg/kg.

In fat all the compounds except the parent (0.02 mg/kg), RH-7968 (0.04 mg/kg) and the glucuronides (0.01 mg/kg) were at levels below 0.01 mg/kg (Jameson, 1989a; Predmore, 1990; Sharma 1992a).

The conversion of the two isomers of the iminolactones RH-6468 to the corresponding isomers of the lactones RH-9129 and RH-9130 during the analysis of cow liver samples was demonstrated. Samples fortified with RH-6468 were processed by the analytical method of Filchner (1994) and the conversion was shown to occur during clean-up on silica gel and C-18 SPE columns (Staurowsky and Wu, 1994).

The manufacturer has proposed the metabolic pathways in lactating goats shown in Figure 2.

Figure 2. Metabolic pathways of fenbuconazole in lactating goats (Batra, 1997).




Hens. Thirty five laying hens each received seven daily doses of [$^{14}$C]fenbuconazole at rates equivalent to 100 ppm in the feed, ten with the phenyl label and 25 with the triazole. The hens were killed 24 hours after the last dose. Samples were analysed by LSC after combustion, and were extracted with methanol and then partitioned into ethyl acetate, n-butanol and water. The resulting organic and aqueous phases were analysed by TLC and HPLC.

The overall recoveries of radioactivity were 94.9-99.4% from the phenyl label and 87.1-95.8% from the triazole label. Excretion accounted for 93.4-97.8% of the phenyl label and 85.1-93.8% of the triazole label, with only 0.4-0.5% of the phenyl label and 0.6-0.7% of the triazole label in the eggs and 0.6% of the phenyl label and 0.6-0.8% of the triazole label in the tissues.

The total $^{14}$C in the eggs reached levels after 6 days of 2.0 mg/kg expressed as fenbuconazole with the phenyl label and 2.7 mg/kg with the triazole label. The major components identified in the eggs were the parent (0.9 mg/kg), its lactone (0.6 mg/kg) and,

from the triazole label, RH-7968 and triazole (0.54 mg/kg). Four other metabolites were identified at levels below 0.2 mg/kg.

Table 5. Fenbuconazole and its metabolites in eggs.

| Compound | $^{14}$C, mg/kg as fenbuconazole | |
|---|---|---|
| | Phenyl label | Triazole label |
| Fenbuconazole | 0.89 | 0.88 |
| Lactones | 0.56 | 0.61 |
| Iminolactones | 0.16 | 0.16 |
| Phenol | 0.10 | 0.10 |
| Benzylic sulfates | 0.02 | 0.09 |
| Triazole | - | 0.54 |
| RH-7968 | 0.08 | 0.77 |
| Benzylic glucuronides | 0.05 | 0.19 |
| Total | 1.86 | 2.61 |

The total $^{14}$C residues in the tissues expressed as fenbuconazole equivalent from the phenyl label were 0.20 mg/kg in muscle, 1.04 mg/kg in the fat, 3.00 mg/kg in the kidneys and 11.6 mg/kg in the liver. The triazole label yielded 0.76 mg/kg in muscle, 0.96 mg/kg in the fat, 2.83 mg/kg in the kidneys and 11.1 mg/kg in the liver.

The highest residues in the liver were the glucuronides (3.69 mg/kg) and triazole 1.25 mg/kg). The parent and seven other metabolites were identified at levels below 1 mg/kg.

The two major components identified in muscle were the triazole (0.28 mg/kg) and triazolylalanine (0.06 mg/kg). The other identified compounds were at levels below 0.05 mg/kg.

In fat fenbuconazole (0.43 mg/kg) and the lactones (0.17 mg/kg) were the main residues. The other metabolites were at levels of <0.01-0.09 mg/kg (Jameson, 1989b; Sharma, 1992b, 1994a).

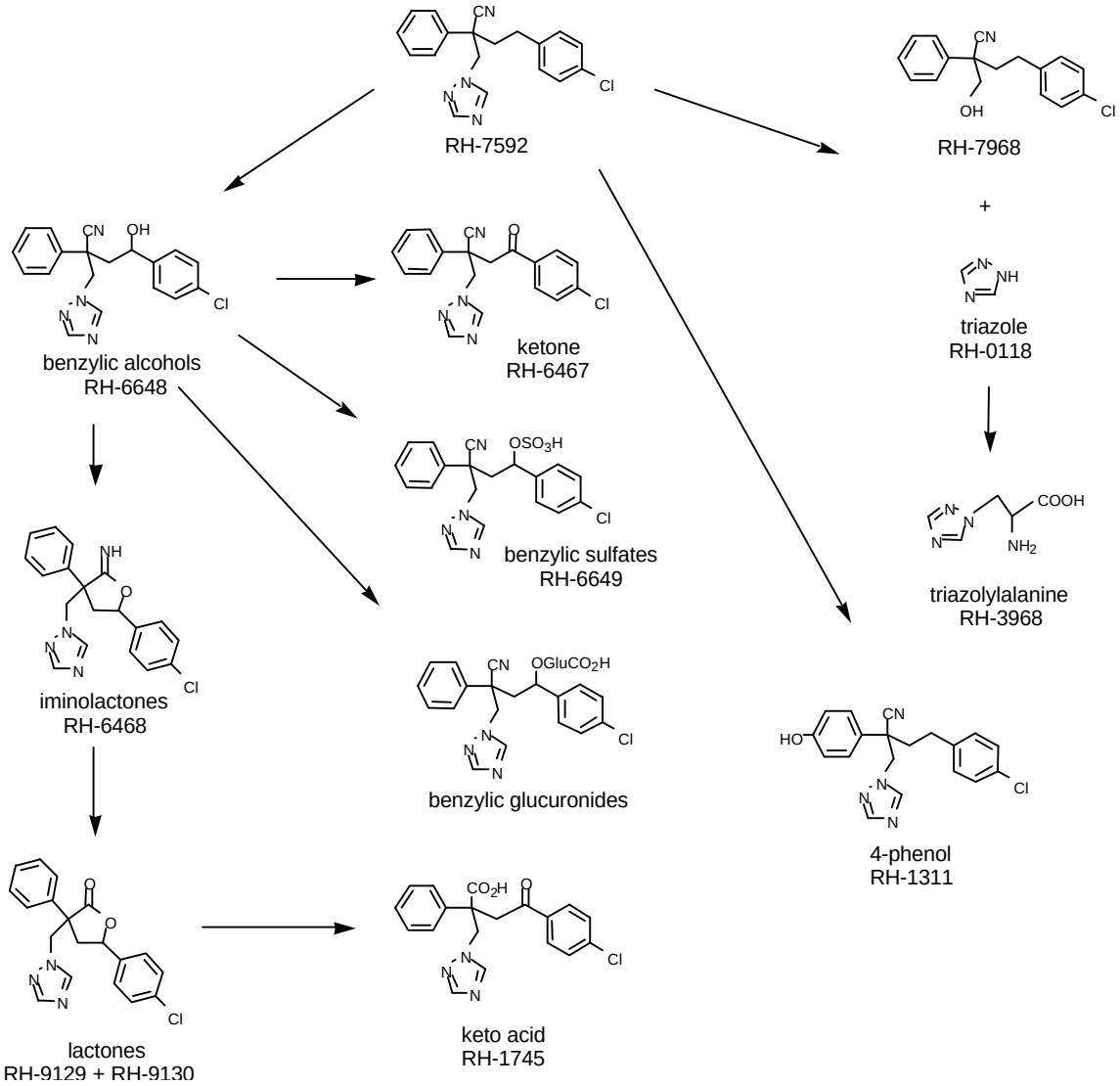
Table 6 shows the averages of the residues from the two labels.

Table 6. Fenbuconazole and its metabolites in hen tissues.

| Compound | $^{14}$C, mg/kg as fenbuconazole | | |
|---|---|---|---|
| | Liver | Muscle | Fat |
| Fenbuconazole | 0.27 | 0.03 | 0.43 |
| Lactones | 0.90 | 0.02 | 0.17 |
| Phenol | 0.97 | 0.02 | <0.01 |
| Iminolactones | 0.97 | 0.02 | 0.06 |
| Ketoacid | 0.32 | <0.01 | <0.01 |
| Benzylic sulfates | 0.64 | 0.03 | <0.01 |
| Triazole | 1.25 | 0.28 | <0.01 |
| Triazolylalanine | <0.01 | 0.06 | <0.01 |
| RH-7968 | 0.87 | <0.01 | 0.09 |
| Benzylic glucuronides | 3.69 | 0.02 | 0.07 |
| Total | 9.26 | 0.52 | 0.98 |

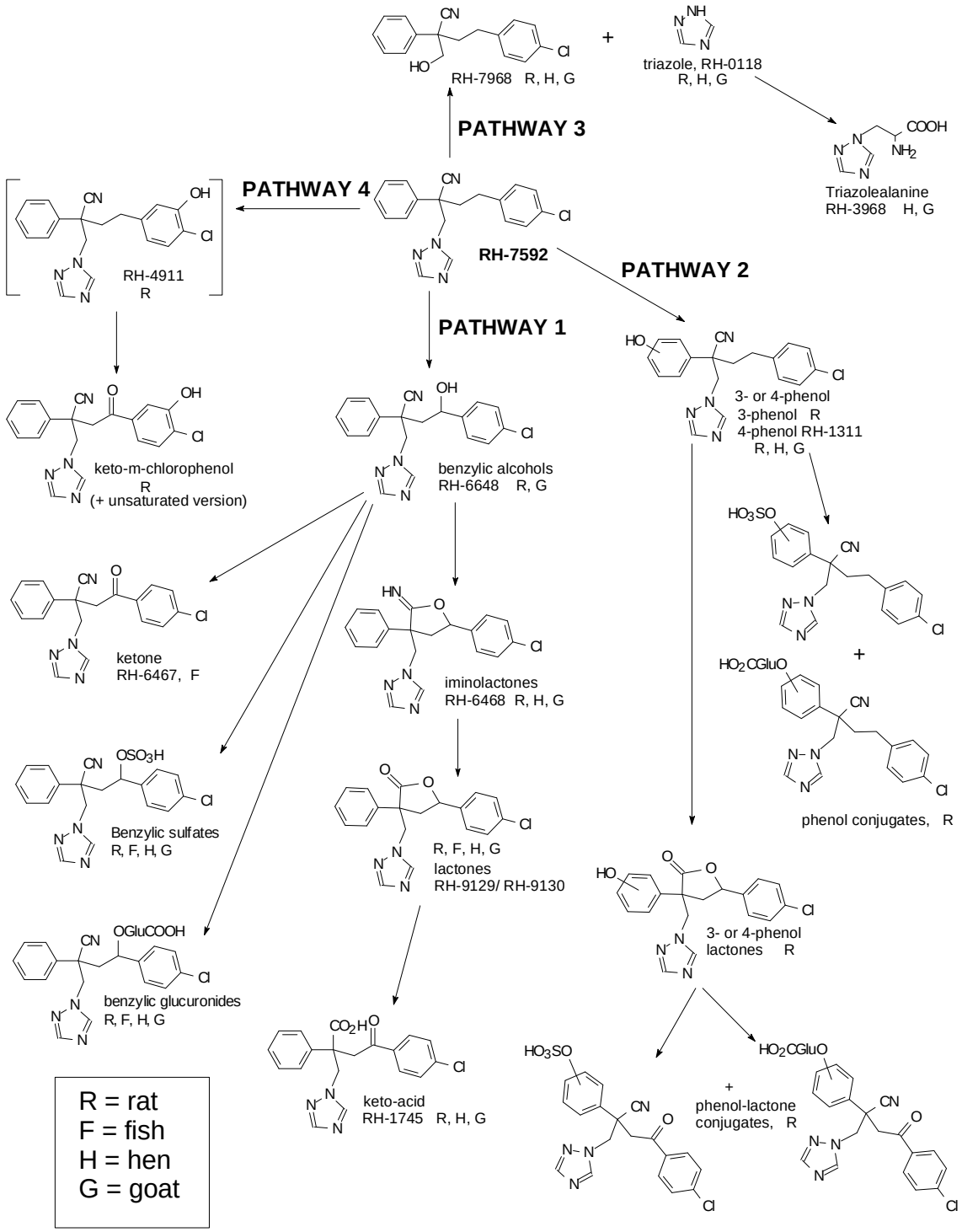
The metabolic pathways for fenbuconazole in laying hens proposed by the manufacturer are shown in Figure 3.

Figure 3. Metabolic pathways of fenbuconazole in laying hens (Batra,1997).




Fenbuconazole appeared to be extensively metabolised in all the animal species studied. Residues have been found which result from four metabolic pathways. The first is oxidation at the benzylic carbon linked to the chlorophenyl ring to produce the isomeric benzylic alcohols and their sulfate and glucuronide conjugates, the iminolactones, lactones, and the keto acid. The second pathway is oxidation of the unchlorinated benzene ring to produce the 3- and 4-phenols and their conjugates. Combinations of paths 1 and 2 produce phenol-lactones and their conjugates. The third path is cleavage of the triazole moiety which produces free triazole and its conjugates, and the alcohol RH-7968. The fourth pathway consists in hydroxylation of the chlorophenyl ring in the 3- position, followed by oxidation to the ketone of the chlorophenol and elimination of HCN to yield the α-unsaturated ketone. An overview of the metabolism of fenbuconazole in animals is shown in Figure 4.

Figure 4. Overview of the metabolic pathways of fenbuconazole in animals (Costlow 1997a; Batra 1997; Ross 1997b).




**Plant metabolism**

Wheat. In a study carried out in the USA (Pennsylvania) in 1987 phenyl- and triazole-labelled fenbuconazole (radiochemical purities 99.0 and 98.5%) were applied as emulsifiable concentrates twice to field-grown winter wheat at 0.4 kg ai/ha 218 and 226 days after sowing (growth stages 45 and 55). Samples were taken at harvest, 39 days after the last treatment. All samples were analysed by LSC after combustion, and were also soxhlet-extracted with

methanol. Straw and chaff were further extracted with sodium hydroxide/methanol, and grain with HC1/methanol. The extracts were evaporated to dryness and reconstituted in ethyl acetate/water and the aqueous layers were partitioned with butanol, chloroform and hexane. The resulting organic and aqueous phases were analysed by TLC, HPLC and GC-MS. The unextractable material was combusted and analysed by LSC.

The total $^{14}C$ residues at harvest (expressed as fenbuconazole equivalent) were 0.44 mg/kg in the grain, 10.6 mg/kg in the straw and 6.1 mg/kg in the chaff from the phenyl label, and 0.14 mg/kg in the grain, 9.8 mg/kg in the straw and 6.1 mg/kg in the chaff from the triazole. On re-analysis of the samples after drying and freezer storage before extraction, the residues found were 0.05 mg/kg in the grain, 18.3 mg/kg in the straw and 7.6 mg/kg in the chaff from the phenyl label, and 0.53 mg/kg in the grain, 13.5 mg/kg in the straw and 6.4 mg/kg in the chaff from the triazole label.

The percentage recoveries of radiolabelled material in the separate extracts are shown in Table 7 and the identities of components of the residues in Table 8. Five components were identified. In the grain the two major compounds identified with the triazole label were triazolylalanine (0.25 mg/kg) and triazolylacetic acid (0.11 mg/kg); the parent was detected, but at less than 0.01 mg/kg. In the straw and chaff the three components identified were fenbuconazole, the lactone and the ketone at respective levels of 8.8-11.8, 1.1-1.4 and 0.59-0.62 mg/kg in the straw, and 3.7-4.5, 0.45-0.49 and 0.16-0.19 mg/kg in the chaff (all residues expressed as fenbuconazole).

Because two sugar conjugates of the chlorophenol RH-4911 were identified in peanut vines in another metabolism study the straw from the wheat treated with the triazole label was re-examined. After soxhlet extraction with methanol the extract was evaporated to dryness, re-constituted in water and the aqueous solution partitioned with ethyl acetate and butanol. The ethyl acetate extract was then partitioned with chloroform to remove the parent compound and the lactone metabolite, and the resulting ethyl acetate and butanol extracts were analysed by TLC. One of the two sugar conjugates, identified as the glucoside of RH-4911, was present at a level of 0.43 mg/kg expressed as fenbuconazole (Hawkins 1989, 1994; Sharma, 1993b).

Table 7. Distribution of extractable radioactivity in wheat at harvest.

| Extract | $^{14}C$, % of total in sample | | | | | |
|---|---|---|---|---|---|---|
| | Grain | | Straw | | Chaff | |
| | Phenyl label | Triazole label | Phenyl label | Triazole label | Phenyl label | Triazole label |
| Soxhlet | | | | | | |
| Ethyl acetate | 25.3 | 5.4 | 85.5 | 79.8 | 69.2 | 77.6 |
| Butanol | 15.4 | 6.8 | 2.4 | 1.6 | 6.6 | 1.8 |
| Aqueous | 4.9 | 32.6 | 0.4 | 1.0 | 0.2 | 0.9 |
| Blender | | | | | | |
| Ethyl acetate | {11.6 | 1.1 | - | - | - | - |
| Aqueous | { | 45.5 | - | - | - | - |
| Base | | | | | | |
| Hexane | - | - | 0.7 | - | - | - |
| Chloroform | - | - | 1.1 | 1.5 | 3.2 | - |
| Ethyl acetate | - | - | 4.1 | 6.0 | 2.8 | 3.7 |
| Butanol | - | - | 1.2 | 1.8 | 2.8 | - |
| Aqueous | - | - | 4.9 | 5.7 | 2.7 | 8.9 |
| Unextractable | 43.7 | 6.9 | - | 2.8 | 12.1 | 7.2 |
| Total | 100.9 | 98.3 | 100.3 | 100.2 | 99.6 | 100.1 |

Table 8. Distribution of fenbuconazole and its identified metabolites in wheat.

| Compound | $^{14}$C, % of total in sample (mg/kg as fenbuconazole) | | | | | |
|---|---|---|---|---|---|---|
| | Phenyl label | | | Triazole label | | |
| | Grain | Straw | Chaff | Grain | Straw | Chaff |
| Fenbuconazole | 12.4(<0.01) | 64.9 (11.8) | 58.6 (4.49) | 1.4 (<0.01) | 60.2 (8.81) | 57.9 (3.67) |
| Lactone | 1.6 (<0.01) | 7.7 (1.40) | 6.5 (0.49) | 0.1 (<0.01) | 7.5 (1.10) | 7.1 (0.45) |
| Ketone | 0.0 (<0.01) | 3.2 (0.59) | 2.2 (0.16) | 0.0 (<0.01) | 4.2 (0.62) | 3.0 (0.19) |
| Triazolylalanine | - | - | - | 48.4 (0.25) | - | - |
| Triazolylacetic acid | - | - | - | 20.1 (0.11) | - | - |

Wheat and grapes. In a laboratory study of uptake and translocation under ambient temperatures and fluorescent lighting, wheat (Fielder variety) and grape plants (Delaware variety) were grown hydroponically in Hoagland nutrient solution for 14 days. Plants at the 3 to 4 leaf growth stage were treated with phenyl-labelled fenbuconazole (radiochemical purity >98%) either through the roots by adding 2.55 mg/l fenbuconazole to the solution or by applying 1 µl of 0.8% EC formulated fenbuconazole to one of the leaves of the plant with a microcapillary tube. Samples were taken at intervals for 14 days. The whole plants were autoradiographed and the combusted foliage and roots, the residual nutrient solution and the root washings were radioassayed. The results are given in Table 9. TLC analyses indicated that the test substance was not degraded significantly. The assays and autoradiograma indicated that uptake from the solution through the roots to the aerial plant parts was rapid, and that the radioactivity migrated rapidly to the tip of the leaf which had been treated. There was evidence of movement of the radioactivity downwards and to adjacent leaves from day 5 in wheat. The same pattern of movement was observed in grapes, but was generally slower. The radioactivity was shown to be highly mobile in the xylem and slightly mobile in the phloem (Sharma, 1993a).

Table 9. Distribution of radioactivity in wheat and grape plants grown hydroponically after treatment of roots or leaves with phenyl-labelled [$^{14}$C]fenbuconazole.

| Crop | Days | $^{14}$C, mg/kg as fenbuconazole | | | | | |
|---|---|---|---|---|---|---|---|
| | | Root treatment | | | Leaf treatment | | |
| | | Foliage | Roots | Nutrient solution | Root | Treated leaf | Untreated leaf |
| Wheat | 1 | 173 | 867 | 1.49 | 0.06 | 85 | 0.7 |
| | 3 | 302 | 940 | 0.69 | 0.13 | 223 | 3.1 |
| | 5 | 352 | 208 | 0.25 | | | |
| | 7 | 269 | 319 | 0.15 | 0.36 | 137 | 3.8 |
| | 14 | 301 | 425 | 0.09 | 0.13 | 203 | 2.5 |
| Grapes | 1 | 1 | 938 | 1.95 | 0.05 | 167 | 0.08 |
| | 3 | 37 | 596 | 0.51 | 0.05 | 90 | 0.7 |
| | 5 | 112 | 219 | 0.25 | 0.16 | 119 | 1.9 |
| | 7 | 53 | 726 | 0.21 | 0.17 | 104 | 0.9 |
| | 14 | 51 | 284 | 0.14 | 0.54 | 107 | 1.3 |

Peanuts. In a field experiment in 1990 in North Carolina, peanuts (Florigiant variety) were treated four times at monthly intervals with either phenyl- or triazole-labelled fenbuconazole (formulated as 6% EC formulations) at 0.57 kg ai/ha. The field plots were irrigated throughout the study. Nuts (separated into kernels and shells) and vines were harvested 28 days after the last treatment. Each sample was ground to a fine powder and radioassayed after combustion to determine the total radioactive residues (Table 10).

Table 10. Total radioactive residues in peanuts and vines after treatment with either phenyl- or triazole-labelled fenbuconazole.

| Sample | $^{14}$C, mg/kg as fenbuconazole | |
|---|---|---|
| | Phenyl label | Triazole label |
| Vine | 13.68 | 13.49 |
| Shells | 1.04 | 1.30 |
| Kernels | 0.064 | 3.98 |

Each sample was extracted with methanol/water. Vines and shells were further extracted with KOH/methanol and kernels also with HCl/methanol. The total extraction efficiencies were >88% for all samples except kernels with the phenyl label from which only 44% of the initial activity could be extracted even after treatment with both acid and base.

Vine extracts were analysed by TLC using various solvent systems and the spots were autoradiographed to identify metabolites by comparison with reference standards. Some extracts were purified on C-18 columns and by TLC and HPLC. The structures of the sugar conjugates were established by β-glucosidase and acid hydrolysis, acetylation, and examination of the products by mass spectrometry. The shell extracts were passed through C-18 columns which were eluted with water and acetonitrile. The acetonitrile fraction was co-chromatographed with standards by TLC.

Because of the low levels of residue in the kernels from the phenyl label compared with the triazole label, it was postulated that the kernels would contain only polar residues. When the kernels were further extracted with KOH/methanol/water there was no further release of radioactivity. It was concluded that the remaining 56% of the phenyl-labelled residue was bound. The extractable residue was reconstituted in water and partitioned with ethyl acetate and butanol. These extracts were shown to contain similar components to those from the conjugates found in the vines.

The extractable triazole-labelled residues in the kernels and shells were analysed by TLC and cation-exchange column chromatography (which separated triazolylacetic acid and triazolylalanine.

The distributions of the identified compounds are shown in Table 11.

Table 11. The nature of radioactive residues in peanuts treated with triazole-labelled fenbuconazole.

| Compound | $^{14}$C, % of TRR and mg/kg as fenbuconazole | | | | | |
|---|---|---|---|---|---|---|
| | Vine | | Shell | | Kernel | |
| | % | Mg/kg | % | mg/kg | % | mg/kg |
| Fenbuconazole | 48.3 | 6.73 | 33.5 | 0.45 | 0 | <0.002 |
| Lactone A, RH-9129 | 4.48 | 0.62 | 0.97 | 0.013 | 0 | <0.002 |
| Ketone, RH-6467 | 8.82 | 1.23 | 7.51 | 0.10 | 0 | <0.002 |
| Iminolactones, RH-6468 | 0 | 0 | 2.45 | 0.033 | 0 | <0.002 |
| Sugar conjugates of RH-4911 | 23.8 | 3.32 | 17.2 | 0.23 | 0.50 | 0.019 |
| (glucoside and malonylglucoside) | | | | | | |
| Triazolylalanine, RH-3968 | 6.57 | 0.91 | 22.2 | 0.30 | 91.8 | 3.50 |
| Triazolylacetic acid, RH-4098 | 0 | 0 | 4.24 | 0.057 | 1.9 | 0.07 |
| Unknown | 2.08 | 0.028 | 1.36 | 0.052 | - | - |
| Bound | 8.00 | 1.11 | 9.9 | 0.13 | 4.4 | 0.17 |

The identified compounds accounted for 88-94% of the total radioactivity recovered from all of the samples except the kernels containing the phenyl label.

The vines and shells contained mainly compounds in which the framework of the original molecule remained intact, namely fenbuconazole, small quantities of the lactone RH-9129, the ketone RH-6467 and the iminolactones RH-6468, and larger quantities of the sugar conjugates (mainly glucoside with some malonylglucoside) of the phenol RH-4911. The kernels contained traces of these but the main residue consisted of triazolylalanine and triazolylacetic acid in which only the triazole ring remained (Sharma, 1992c, 1993b).

Peaches. A study was carried out in the USA (Pennsylvania) in 1987. Phenyl- and triazole-labelled fenbuconazole (radiochemical purities 98.5 and 98.8%), formulated as emulsifiable concentrates were sprayed five times on outdoor peach trees, from blossom until 22 days before harvest (no rainfall on the days of application, except the first where 3.8mm fell, average temperature 21°C), at a rate of 0.2 kg ai/ha (0.7 for apples). Samples were taken at harvest and analysed by LSC after combustion. The samples were also extracted with methanol in Soxhlets, 'polytrons' and blenders. The extracts were evaporated to dryness and reconstituted in chloroform/water, and the resulting aqueous layers were partitioned with ethyl acetate, ether and butanol. The organic and aqueous phases were analysed by TLC, HPLC and GC-MS. The unextractable material was combusted and analysed by LSC.

The total $^{14}C$ residues in the peaches at harvest (expressed as fenbuconazole) were 0.08 mg/kg from the phenyl label and 0.12 mg/kg from the triazole label.

The distributions of the radiolabels are shown in Tables 12 and 13. The two main components of the residue were triazolylalanine and the parent compound, which were present at levels of 0.06 and 0.02-0.04 mg/kg (as fenbuconazole) respectively. The lactone and triazolylacetic acid were also identified. Five unknown compounds were present at levels below 0.01 mg/kg.

TLC of the ethyl acetate extract showed that a component of the residue was similar to the conjugates of RH-4911 found in peanuts. Co-chromatography with the conjugates identified in the peanut metabolism study showed that they were identical (Hawkins, 1988; Sharma, 1993b).

Table 12. Distribution of extractable radioactivity in peaches at harvest.

| Extract | % of extractable $^{14}C$ in fruit | |
|---|---|---|
| | Phenyl label | Triazole label |
| Chloroform | 70.5 | 21.5 |
| Ethyl acetate | 15.7 | - |
| Ether | - | 0.3 |
| Butanol | 5.8 | 7.9 |
| Aqueous | 1.6 | 64.6 |
| Unextractable | 6.4 | 4.6 |
| Total | 100.0 | 98.9 |

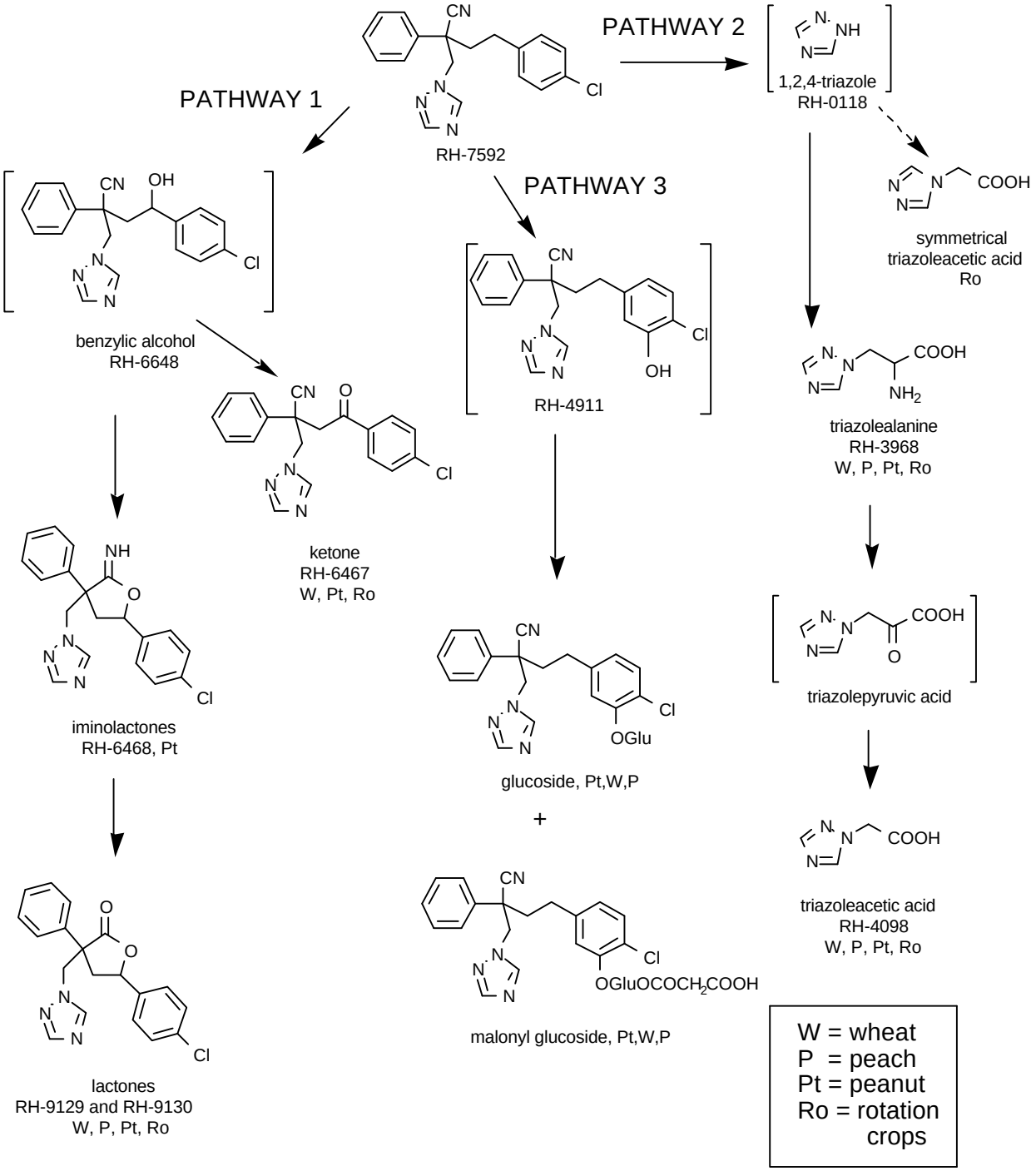
Table 13. Fenbuconazole and its metabolites in fruit at harvest.

| Compound | $^{14}C$, % of TRR (mg/kg as fenbuconazole) | |
|---|---|---|
| | Phenyl label | Triazole label |
| Parent | 45.0 (0.04) | 15.5 (0.02) |
| Lactone | 14.2 (0.01) | 4.3 (<0.01) |
| Triazolylalanine | - | 47.5 (0.06) |

| Compound | $^{14}$C, % of TRR (mg/kg as fenbuconazole) | |
|---|---|---|
| | Phenyl label | Triazole label |
| Sugar conjugates of RH-4911 | - | 4.4 (<0.01) |
| Triazolyl acetic acid | - | 6.7 (<0.01) |

A further study on sugar beet in 1997 was referenced by the manufacturer but not submitted for review. The manufacturer has proposed the metabolic pathway in plants shown in Figure 5.

Figure 5. Metabolic pathways of fenbuconazole in plants (Costlow, 1997a).



**Metabolism and distribution in rotational crops**

In a field trial in 1987 in the USA (Pennsylvania) wheat, turnips and collards were grown in bare soil treated with either phenyl- or triazole-labelled fenbuconazole (formulated as emulsifiable concentrate, radiochemical purities >98.5%) at a rate of 8.96 kg ai/ha. The crops were planted 30, 99 and 365 days after application and sampled at various times up to harvest. The samples were analysed by LSC after combustion, and extracted as in the wheat metabolism study. The resulting organic and aqueous phases were analysed by TLC and HPLC. The unextractable material was combusted and the $^{14}C$ determined by LSC.

The distribution of radioactivity in the plants is shown in Table 14.

Table 14. Radioactive residues from phenyl- and triazole-labelled fenbuconazole in rotational crops following application to bare soil.

| Sample | Days between application and planting | Days between planting and sampling | $^{14}C$, mg/kg (as fenbuconazole) | |
|---|---|---|---|---|
| | | | Phenyl label | Triazole label |
| Collards | 30 | 47 | 0.43 | 4.3 |
| | | 56 | 0.32 | 5.5 |
| | | 83 | 0.19 | 7.4 |
| | 99 | 52 | 0.59 | 15.0 |
| | | 61 | 0.38 | 18.0 |
| | | 88 | 0.26 | 25.0 |
| | 365 | 77 | 0.24 | 37.0 |
| | | 94 | 0.30 | 28.0 |
| | | 132 | 0.24 | 17.0 |
| Turnips | | | | |
| Tops/Roots | 30 | 31 | 1.78/- | 4.1/- |
| | | 56 | 0.78/0.48 | 3.5/3.4 |
| | | 75 | 0.86/0.39 | 16.0/5.1 |
| | 99 | 52 | 0.45/- | 11.0/- |
| | | 61 | 0.42/0.31 | 25.0/58.0 |
| | | 88 | 0.36/0.10 | 30.0/18.0 |
| | 365 | 77 | -/- | 34.0/- |
| | | 94 | -/- | 20.0/- |
| | | 152 | 0.21/0.29 | 6.9/4.8 |
| Wheat | | | | |
| Straw = S | 30 | 258 | 0.63(S)/0.30(H) | 5.7(S)/14.0(H) |
| Head = H | | 273 | 1.4(S)/0.69(H) | 11.0(S)/17.0(H) |
| Grain = G | | 281 | 1.6(S)/0.78(C)/0.52(G) | 14.0(S)/13.0(C)/29.0(G) |
| Chaff = C | 99 | 314 | 49.0(S)/0.57(C)/0.46(G) | 47.0(S)/70.0(C)/122.0(G) |
| | 365 | 282 | 0.5(S)/0.92(H) | 6.6(S)/16.0(H) |
| | | 302 | 0.58(S)/1.4(H) | 7.4(S)/21.0(H) |
| | | 318 | 1.2(S)/0.88(C)/2.3(G) | 9.1(S)/8.2(C)/43.0(G) |

In collards the total $^{14}C$ from the phenyl label residue expressed as fenbuconazole at harvest increased slightly from 0.19 mg/kg in crops grown in soil aged for 30 days to 0.24 mg/kg in crops grown in soil aged for 365 days, and that from the triazole label from 7.4 to 17 mg/kg (25 mg/kg for crops grown in soil aged for 99 days).

The total $^{14}C$ from the phenyl label decreased in turnip tops at harvest from 0.86 mg/kg from the 30-day planting to 0.21 mg/kg from the 365-day, and in the roots from 0.39 to 0.29 mg/kg. The $^{14}C$ levels from the triazole label at harvest were 16.0, 30.0 and 6.9 mg/kg in the tops and 5.1, 18.0 and 4.8 mg/kg in the roots from the 30-, 99- and 365-day plantings.

In wheat the total $^{14}C$ at harvest from the phenyl label was equivalent to 1.6, 49 and 1.2 mg/kg in the straw and 0.52, 0.46 and 2.3 mg/kg in the grain from the successive plantings. The corresponding levels from the triazole label were 14, 47 and 9.1 mg/kg in the

straw and 29, 122 and 43 mg/kg in the grain. The manufacturer stated that the high levels in the wheat planted 99 days after treatment were possibly due to the low yield and near crop failure two weeks before harvest.

The recoveries of $^{14}C$ from the triazole label are shown in Table 15. Only samples at harvest with this label were completely analysed: analysis of selected samples with the phenyl label did not show any compound that was not seen with the triazole label. Two major metabolites were identified as triazolylacetic acid and triazolylanine, which accounted respectively 4.4-19.0 and 68-76% of the total radioactivity in collards, 19-21 and 58-62% in turnip tops, 1.7-7.8 and 81-90% in turnip roots, 27-34 and 56-65% in wheat grain, and 49-68 and 8.1-29% in wheat straw. Three other components were identified as the parent compound and the ketone and lactone metabolites, none of which accounted for more than 4% of the total radioactivity in any of the crops (the highest residue was 0.45 mg/kg fenbuconazole in wheat straw). The distributions of the identified compounds are shown in Table 16 (O'Dowd, 1990b; Hawkins, 1992).

Table 15. Distribution of $^{14}C$ at harvest in rotational crops treated with thiazole-labelled fenbuconazole.

| Sample | Days between application and planting | $^{14}C$, % of TRR | | |
|---|---|---|---|---|
| | | Ethyl acetate phase | Aqueous phase | Unextractable |
| Collards | 30 | 2.3 | 91.6 | 6.1 |
| | 99 | 1.0 | 92.1 | 6.9 |
| | 365 | 0.8 | 91.1 | 8.1 |
| Turnips | 30 | 3.0/2.7 | 83.8/91.6 | 13.2/5.7 |
| Tops/Roots | 99 | 1.1/0.7 | 89.7/96.1 | 9.2/3.2 |
| | 365 | 1.1/0.9 | 84.9/92.0 | 14.0/7.1 |
| Wheat | | | | |
| Grain/Straw | 30 | 0.2/9.6 | 94.8/87.9 | 5.0/2.5 |
| | 99 | 0.3/2.7 | 94.5/92.2 | 5.2/5.1 |
| | 365 | 0.1/4.5 | 94.5/87.0 | 5.3/8.5 |

Table 16. Distribution of identified compounds in residues from treatments with triazole-labelled tebuconazole.

| Sample | Days between application and planting | $^{14}C$, % of TRR and (mg/kg as fenbuconazole) | | | | |
|---|---|---|---|---|---|---|
| | | Parent | Lactone | Ketone | TAA | TA |
| Collards | 30 | 0.8 (0.06) | - | - | 4.4 (0.62) | 76.0 (5.6) |
| | 99 | - | - | - | 18.5 (4.6) | 67.7 (17.0) |
| | 365 | - | - | - | 14.4 (2.4) | 71.4 (12.0) |
| Turnips | | | | | | |
| Tops | 30 | 1.6 (0.24) | 1.2 (0.17) | 0.4 (0.06) | 18.8 (2.8) | 58.3 (8.8) |
| | 99 | - | - | - | 21.3 (6.6) | 62.4 (19.0) |
| | 365 | - | - | - | 19.6 (1.4) | 60.7 (4.2) |
| Roots | 30 | 1.5 (0.08) | 0.4 (0.02) | - | 2.6 (0.13) | 83.9 (4.3) |
| | 99 | - | - | - | 1.7 (0.31) | 89.8 (16.0) |
| | 365 | - | - | - | 7.8 (0.37) | 80.9 (3.9) |
| Wheat | | | | | | |
| Grain | 30 | - | - | - | 26.8 (7.8) | 64.8 (19.0) |
| | 99 | - | - | - | 31.8 (39.0) | 57.6 (70.0) |
| | 365 | - | - | - | 34.2 (15.0) | 55.8 (24.0) |
| Straw | 30 | 3.4 (0.45) | 0.9 (0.11) | 0.5 (0.06) | 55.5 (7.8) | 26.0 (3.6) |
| | 99 | - | - | - | 68.1 (32.0) | 8.1 (3.8) |
| | 365 | 0.2 (0.05) | 0.2 (0.03) | - | 49.0 (4.4) | 29.2 (2.7) |

TAA: triazoleylacetic acid
TA: triazolylalanine

Two further studies were carried out with phenyl-labelled fenbuconazole (radiochemical purities >99% and >96%). In one a 3% EC formulation was applied to sandy loam soil at 4 x 0.28 kg ai/ha. Lettuce (Buttercrunch variety), radishes (White icicle), and sorghum (Pioneer 8222) were planted 210 days after treatment and harvested at maturity (lettuce and radishes 291 days, sorghum 399 days after treatment).

In the other trial a 2% EC formulation was applied to sandy loam soil at 3 x 0.07 kg ai/ha. Lettuce (Waldmans Greenleaf), carrots (Imperator 58) and barley (BB82-425) were planted 35 and 260 days after treatment. Samples of lettuce and barley from the 35-day plantings were taken 133 days after treatment and the three crops were harvested at maturity: lettuce 210, carrots 288 and barley 253 days after treatment (DAT). Barley planted 260 DAT was sampled immediately after treatment, and all three crops were sampled at maturity: 360 DAT for lettuce, 260 DAT for carrots and barley].

All the samples were assayed for $^{14}C$ after combustion. Those with total radioactive residues of >0.01 mg/kg as fenbuconazole were Soxhlet-extracted with methanol and partitioned with sodium chloride and dichloromethane. After clean-up on silica gel, Florisil and C-18 columns the extracts were analysed by GLC. The analyses revealed no quantifiable residues of fenbuconazole (one sample of barley forage showed 0.008 mg/kg) or the metabolites RH-9129, RH-9130 and RH-6467. The limits of determination were 0.01 to 0.05 mg/kg depending on the sample. The samples were therefore analysed by TLC with radiometric detection and co-chromatography with reference standards. The TLC spots were characterized as either “non-polar” (radioactivity in the region of metabolite standards) or “polar” according to their Rf values. The extracts from the above extraction procedure were radioassayed by LSC, and the crop samples remaining after extraction were radioassayed after combustion.

The total radioactive residues and the characteristics of the residue components are given in Tables 17 and 18.

Table 17. Radioactive residues from phenyl-labelled fenbuconazole in rotational crops after application to bare soil at 4 x 0.28 kg ai/ha.

| Sample | TRR, mg/kg as fenbuconazole | Extracted | | Bound | | Extracted, non-polar | | Extracted, polar | |
|---|---|---|---|---|---|---|---|---|---|
| | | % | mg/kg | % | mg/kg | % | mg/kg | % | mg/kg |
| Lettuce | 0.039 | 55 | 0.022 | 41 | 0.016 | 35 | 0.013 | 20 | 0.008 |
| Radish roots | 0.008 | | | | | | | | |
| Radish leaves | 0.033 | 51 | 0.017 | 42 | 0.014 | 27 | 0.009 | 27 | 0.009 |
| Sorghum forage | 0.039 | 79 | 0.030 | 23 | 0.009 | 40 | 0.015 | 39 | 0.015 |
| Sorghum grain | 0.0047 | | | | | | | | |
| Sorghum stover | 0.033 | 28 | 0.009 | 43 | 0.017 | | | | |

After application at 4 x 0.28 kg ai/ha the TRR in all samples was below 0.04 mg/kg. The residues in radish roots and sorghum grain were below 0.001 mg/kg, and in lettuce, radish leaves and sorghum forage between 0.03 and 0.04 mg/kg and sorghum stover residue was 0.033 mg/kg.

Table 18. Radioactive residues from phenyl-labelled fenbuconazole in rotational crops after application to bare soil at 3 x 0.07 kg ai/ha.

| Sample | TRR, mg/kg as fenbuconazole | Extracted | | Bound | | Extracted, non-polar | | Extracted, polar | |
|---|---|---|---|---|---|---|---|---|---|
| | | % | mg/kg | % | mg/kg | % | mg/kg | % | mg/kg |
| 35-day planting | | | | | | | | | |
| Lettuce | 0.004 | | | | | | | | |
| Carrot root | 0.008 | | | | | | | | |
| Carrot leaf | 0.008 | 44 | 0.004 | 38 | 0.003 | | | | |
| Barley forage | 0.016 | 46 | 0.007 | 19 | 0.003 | | | | |
| Barley grain[1] | 0.008 | | | | | | | | |
| Barley straw | 0.019 | 52 | 0.01 | 16 | 0.003 | 38 | 0.007 | 14 | 0.003 |
| 260-day planting | | | | | | | | | |
| Lettuce | 0.009 | 81 | 0.007 | 33 | 0.003 | | | | |
| Carrot root | 0.005 | | | | | | | | |
| Carrot leaf | 0.016 | 69 | 0.011 | 31 | 0.005 | 13 | 0.002 | 61 | 0.009 |
| Barley forage | 0.082 | 73 | 0.06 | 16 | 0.013 | 21 | 0.017 | 52 | 0.042 |
| Barley grain[1] | 0.033 | | | | | | | | |
| Barley straw | 0.162 | 70 | 0.113 | 32 | 0.051 | 23 | 0.037 | 47 | 0.075 |

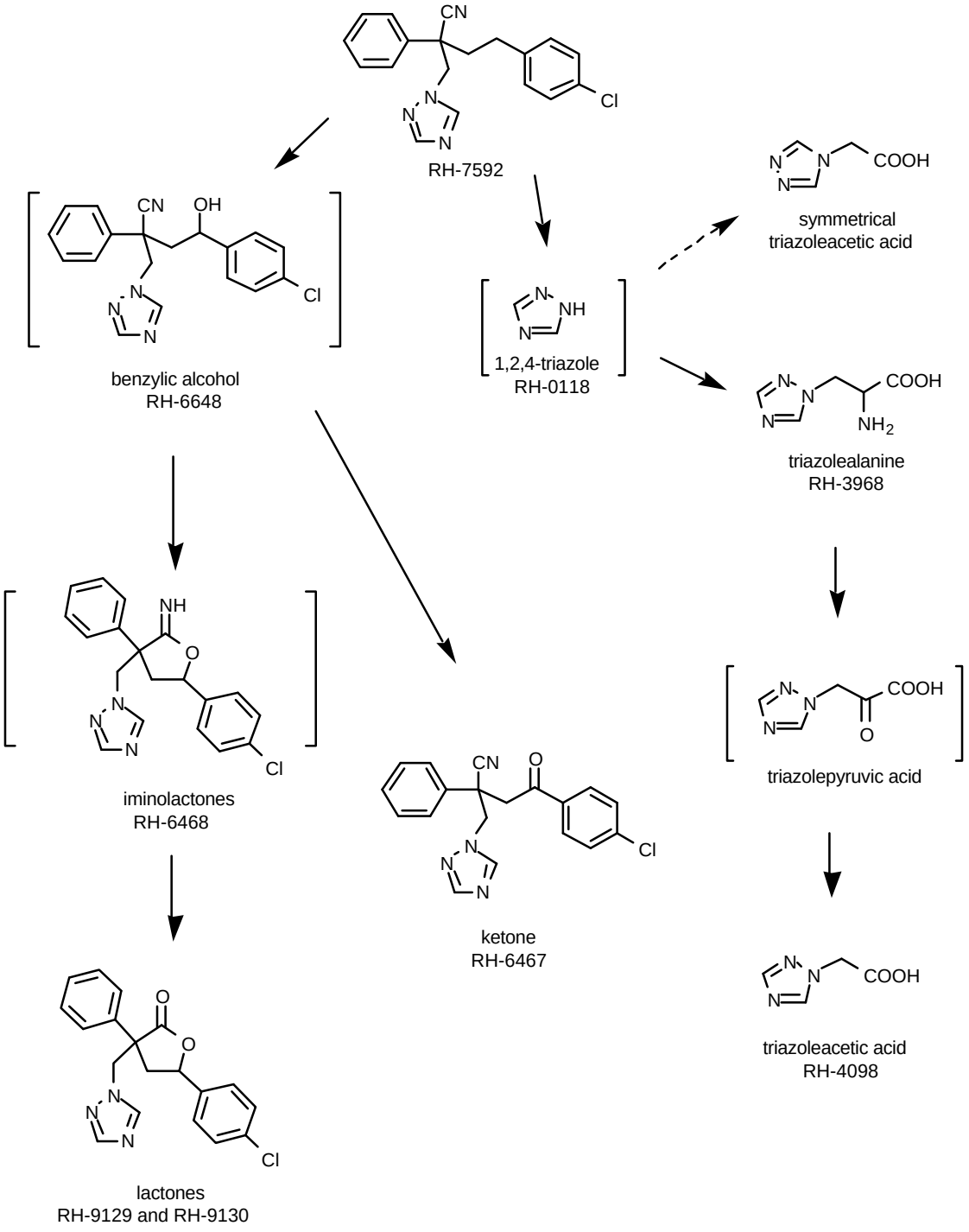
[1] Sample was not sufficient for further analysis

After application at 3 x 0.07 kg ai/ha, the residues in lettuce and carrot roots were below 0.01 mg/kg. The TRR in the grain samples was much lower from the 35-day than the 260-day planting. The company postulated that this may be because fenbuconazole is not translocated well via the roots, whereas degradation products formed in the soil may be taken up by the plants after a planting interval of 260 days. The highest residue found was 0.16 mg/kg, in barley straw. Only two samples (barley straw and forage) in the two studies had residues above 0.05 mg/kg.

TLC analyses confirmed the GLC results. No individual component was found above 0.03 mg/kg equivalents. The non-polar components altogether accounted for 13-38% of the TRR in each sample; the residues were equivalent to 0.002-0.037 mg/kg of fenbuconazole. Another 14-61% of the TRRs was characterized as polar and comprised 2 or 3 components in each sample, the range of residues being equivalent to 0.003-0.076 mg/kg. No single polar component exceeded 0.029 mg/kg fenbuconazole equivalents. The polar components were thought by the manufacturer to be the glucoase conjugates of RH-4911 (Sharma, 1994b).

The manufacturer has proposed the metabolic pathways for fenbuconazole in rotational crops shown in Figure 6 (Batra, 1997).

Figure 6. Metabolic pathways of fenbuconazole in rotational crops




**Environmental fate in soil and water/sediment systems**

Degradation on soil

An aerobic degradation study was conducted according to the German BBA guidelines. Triazole-labelled fenbuconazole (0.1 mg/kg dry soil, radiochemical purity 99.1%, specific activity 835 MBq/g) was added to Itingen III silt loam, Sisseln I sandy loam and Speyer 2.2 sandy loam soil (100 g dry weight) at 40% field capacity. Samples of each soil were

incubated in the dark at 20°C and $CO_2$ and organic volatiles were trapped in NaOH and ethylene glycol respectively. Samples were taken after 0, 5, 7, 14, 33, 50, 70 and 96 days and extracted several times with acetonitrile and after the later times also with acetonitrile/water, and subsequently with methanol by Soxhlet. Radioactivity in liquid samples was measured directly by LSC, soil extracts were analysed by LSC and TLC and the residual radioactivity in the soil after extraction was determined by combustion and LSC.

Over the course of the experiment (96 days) the recovered $^{14}C$ for all the soils was always >95% of the applied radioactivity (AR). Volatile compounds were <0.1% of the AR and the evolved $^{14}CO_2$ increased up to 2.3% of the AR after 96 days (in Sisseln I soil). Up to five degradation products were observed in each of the three soils during the course of the experiment but only one was identified, as RH-6467, and constituted <10% of the AR. One product from the Speyer 2.2 incubation and one from the Sisseln I incubation reached 15% of the AR towards the end of the experiment but it is not clear whether this was the same compound in both cases. No other compound accounted for >10 % of the AR. Unextractable radioactivity reached only 15.1% after 96 days in the Itingen III soil but amounted to 45.5 and 36.3% in the Sisseln I and Speyer 2.2 soils respectively. This was consistent with the half-lives where first order kinetics gave 269 ($r^2$=0.79), 38 ($r^2$=0.93) and 74 ($r^2$=0.96) days for Itingen III, Sisseln I and Speyer 2.2 respectively (Mamouni, 1992).

A second aerobic and anaerobic degradation study was conducted according to US EPA guidelines. [$^{14}C$]Fenbuconazole (radiochemical purity 98 or 98.6%, specific activity 771 or 775 MBq/g) labelled in the phenyl or triazole ring was added to Lawrenceville silty clay loam (200 g, moisture content 15%) or Pasquotank sandy loam (200 g, moisture content 20%) in cellulose at a rate of 1 mg/kg (and at 30 mg/kg to identify degradation products). Some samples were sterilized and all were incubated in the dark at 25°C. After 30 days some samples were purged with nitrogen and then flooded with water to provide anaerobic conditions. $CO_2$ evolved from samples under aerobic conditions was trapped in NaOH. Samples of soil (10 g) incubated under aerobic conditions were taken after 7, 14, 21, 28, 44, 61, 90, 120, 181, 240 and 363 days, and 25-ml samples of slurry were taken 17, 30 and 60 days after the establishment of anaerobic conditions. The samples were extracted with acetonitrile/acetic acid and after later times with NaOH (the anaerobic slurry was first filtered and dried) and analysed by TLC. Overall radioactivity balances were generally 90-105% during the experiment.

The products RH-9129, RH-9130 and RH-6467 were confirmed by TLC, HPLC and GC-MS as present in both the aerobic and anaerobic incubations with both labels. In the aerobic incubations the concentrations of RH-9129, RH-9130 and RH-6467 reached maxima of <10%, <4.5% and <7.9% respectively. In the aerobic incubations with the triazole label free triazole was also identified at levels up to 13.6%. Levels of $^{14}CO_2$ reached 1.5% from the triazole label and 37% from the phenyl label after 365 days. Apart from this there was no difference between the two label positions and both were used to calculate half-lives for fenbuconazole, assuming first-order kinetics. They were as shown below.

| | Lawrenceville (silty clay loam) | Pasquotank (sandy loam) |
|---|---|---|
| Aerobic conditions | 258 days | 367 days |
| Anaerobic conditions | 464 days | 655 days |

No degradation was observed under sterile conditions during the 363 days of the study (Schieber, 1988a).

In a photolysis study according to US EPA guidelines phenyl-labelled fenbuconazole (10 mg/kg, specific activity 753 MBq/g, 96.2% pure) was applied to the surface of a Camden county sandy loam soil layer (2 g) which was maintained at 26°C and irradiated with a xenon

lamp (approx. 150 W/m², of similar intensity to sunlight in New Jersey) for periods of 12 h followed by 12 h darkness for 30 days. Volatile compounds were trapped with polyurethane plugs, KOH and sulfuric acid. Duplicate soil samples were extracted with acetonitrile/acetic acid after 0, 3, 7, 14, 21, 30 days and the extracts analysed by TLC. After extraction the soil was combusted and the unextracted radioactivity quantified by TLC. At all sampling times the overall recovery of radioactivity was >90%.

The levels of trapped volatiles reached 0.02% after 30 days, at which time the unextractable radioactivity reached its maximum of 4%. Fenbuconazole was the only compound identified in the extract and had a half-life of 79 days according to first order kinetics. No degradation observed in the dark control (Wang, 1991a).

**Adsorption and desorption**

In a study according to US EPA guidelines triazole-labelled fenbuconazole (specific activity 775 MBq/g, purity 98%) was dissolved in 0.01M $CaCl_2$ (0.33, 0.22, 0.11 and 0.03 µg/l) and 10-40 ml aliquots were added in duplicate to five different soils (2-2.5 g). The soils were equilibrated for 24 h at 25°C. After centrifugation the supernatant was removed and desorption was measured by equilibrating the soil with fresh 0.01M $CaCl_2$ (10-40 ml) for 72 h. The $^{14}C$ in the solutions was quantified by LSC and that in the soils by combustion and LSC. TLC analysis of the solutions showed that fenbuconazole was the only source of radioactivity. The results are shown in Table 19 (Schieber, 1988c).

Table 19 Freundlich adsorption/desorption coefficients for fenbuconazole in five soils.

| | Adsorption | | | Desorption | | |
|---|---|---|---|---|---|---|
| | n[1] | $K_d$ | $K_{oc}$ | n | $K_d$ | $K_{oc}$ |
| Cecil (clay) | 0.93 | 5.1 | 2185 | 0.95 | 7.1 | 3087 |
| Keeton (loam) | 1.01 | 75.2 | 5402 | 1.01 | 147.7 | 10625 |
| Lakeland (sand) | 1.22 | 7.6 | 2607 | 0.93 | 2.3 | 793 |
| Pasquotank (sandy loam) | 1.00 | 115.4 | 9042 | 0.96 | 132.2 | 10328 |
| Lawrenceville (silty clay loam) | 0.85 | 20.1 | 2884 | 0.87 | 33.0 | 4714 |

[1] Slope of Freundlich adsorption isotherm

Mobility in soil

In a study according to US EPA guidelines Pasquotank sandy loam soil was treated with triazole- or phenyl-labelled fenbuconazole (1 mg/kg, specific activity 775 or 771 MBq/g, purity 98%) and aerobically aged at 25 °C for 30 days. At this time <1% of the AR had been lost as $^{14}CO_2$ and >90% was fenbuconazole. Duplicate columns, 5.5 cm diameter, were filled to a height of 30 cm with untreated Pasquotank soil, wetted from the bottom until saturated and allowed to drain overnight. Aged treated soil (40 g) was added and the columns were leached for 7-14 days with 1000 ml water (42 cm depth). The leachate was assayed for radioactivity by LSC and the soil column divided into segments, extracted with acetonitrile/acetic acid and analysed by LSC and TLC.

The average $^{14}C$ balance at the end of the leaching was 98%. Radioactivity in the leachate accounted for 0.2% of the AR and the leachate was not analysed further. About 97% of the radiolabel was located on the top 6 cm segment of the column and was almost all due to fenbuconazole; trace amounts of RH-9129, RH-9130 and RH-6467 were also identified (Schieber, 1988b)

In a study according to German BBA guidelines sieved air-dried German standard soils (2.1, 2.2 and 2.3) were used to fill duplicate glass columns (5 cm diameter) to a height of 30 cm. The soils were saturated from the top and fenbuconazole (15 µg) was added after

draining. Water (393 ml, equivalent to 200 mm depth) was passed through the columns for two days and the leachate collected. The leachate was analysed by GLC (recovery 76%, limit of detection 0.75 µg/l) but no fenbuconazole was detected in any sample (Specht, 1992).

**Soil dissipation under field conditions**

In a study conducted according to German BBA guidelines fenbuconazole (75 g/ha) was sprayed onto bare soil plots (5 m x 5 m) at four sites in Northern Germany (Klein-Offenseth, Bad Oldesloe, Hamburg and Walsrode) in April 1989. Samples (20 per plot) taken to a depth of 15 cm were bulked at 0, 7, 28, 56, 112, 224, 365 and 504 days after application. The soils were extracted with methanol and after clean-up the extracts were analysed by GLC. The limit of detection was 0.01 mg/kg. In the Bad Oldesloe soil RH-9129 was identified after 8 weeks at 5 µg/kg dry soil, but at no other time was RH-9129, RH-9130 or RH-6467 detectable.

Table 20. Fenbuconazole concentrations in German soils at intervals after field application.

| Days after application | Fenbuconazole, mg/kg dry soil | | | |
|---|---|---|---|---|
| | Bad Oldesloe | Klein-Offenseth | Walsrode | Hamburg |
| Before application | nd | nd | nd | nd |
| 0 | 45 | 29 | 30 | 65 |
| 7 | 42 | 38 | 34 | 23 |
| 48 | 22 | 16 | 17 | 17 |
| 56 | 26 | 19 | 19 | 8 |
| 112 | 13 | 15 | 11 | 11 |
| 224 | 9 | 7 | 18 | 19 |
| 365 | 5 | 6 | 16 | 15 |
| 504 | 10 | 1 | 15 | 13 |

Graphically estimated half-lives for fenbuconazole in these soils were approximately 56, 70, 84 and 28 days. Weather conditions were generally within the 30 year maxima and minima for monthly temperature and precipitation (Bieber *et al.*, 1990).

In a study according to EPA guidelines undertaken at four US locations fenbuconazole was applied twice or five times. At Minnesota (loamy soil) 2 x 0.14 kg/ha were applied to bare soil on 14 and 31 May 1990. At Georgia (loamy sand) 2 x 0.14 kg/ha were applied to bare soil on 19 July and 2 August 1989. At the Northern California site (clay) 5 x 0.22 kg/ha were applied to bare soil on 7 and 21 August, 5 and 21 September and 5 October 1989. At the Southern California site (sandy loam, 5 x 0.22 kg/ha) were applied to wheat on 1, 15 and 29 August and 12 and 26 September.

At all sites there were three treated plots and one control plot (30 x 6 m). Samples were taken at 0, 14, 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 510 and 550 days after the last application. Five samples were taken from the control and each treated plot, divided into 0-15, 15-30, 30-45, 45-60, 60-90 and 90-120 cm sections, (0-7.5 and 7.5-15 cm in Minnesota) and composited. The soils were extracted with methanol and the residues determined by GLC with a quoted limit of determination of 0.01 mg/kg and recoveries of >90%. The residues found are shown graphically in Figure 7, and the calculated DT-50 and DT-90 values in Table 21.

Figure 7. Concentrations of fenbuconazole in 0-15 cm soil layers (USA, 1989-90).

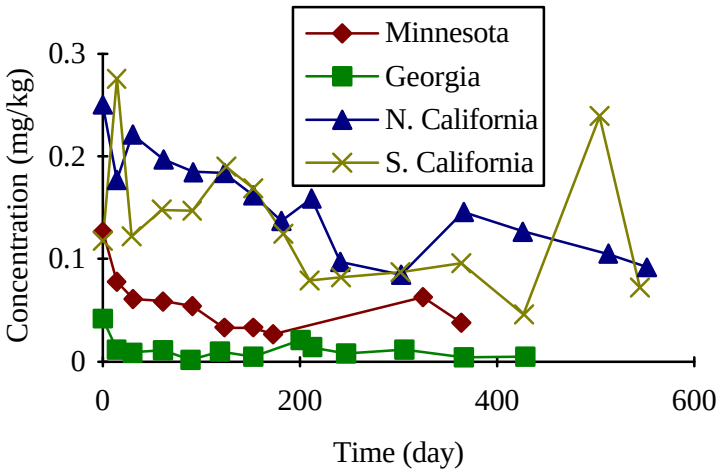



Table 21. DT-50 and DT-90 values for fenbuconazole in 0-15 cm soil layers.

| Location | DT-50, days | DT-90, days |
|---|---|---|
| Minnesota | 30 | >364 |
| Georgia | 10 | 360 |
| N. California | 425 | >550 |
| S. California | 200 | >545 |

RH-9130, RH-9129 and RH-6467 were consistently found in the 0-15 cm layer up to the end of sampling in Southern California at levels up to 0.01, 0.031 and 0.016 mg/kg respectively. RH-9129 was found consistently, but at lower levels, in Northern California where fenbuconazole was regularly found at 15-30 cm at varying concentrations up to 0.021 mg/kg throughout the trial. In the trials in Georgia and Minnesota fenbuconazole was not identified below 15 cm and no degradation products were detected at any depth. The soil temperatures were recorded and are shown in Table 22.

Table 22. Soil temperatures at 5 cm depth averaged over 60-day periods in the US trials.

| Location | Soil temperature, °C | | | | |
|---|---|---|---|---|---|
| | 0-60 days | 60-120 days | 120-180 days | 180-240 days | 240-300 days |
| Minnesota | 23.3 | 21.9 | 5.7 | -2.8 | -2.4[1] |
| Georgia[2] | 30.6 | 19.4 | 10.6 | 16.7 | 24.4 |
| N. California | 12 | 7.2 | 10.9 | 19.7 | 22.9[1] |
| S. California | 16.5 | 7.7 | 12.2 | 22.2 | 35.8 |

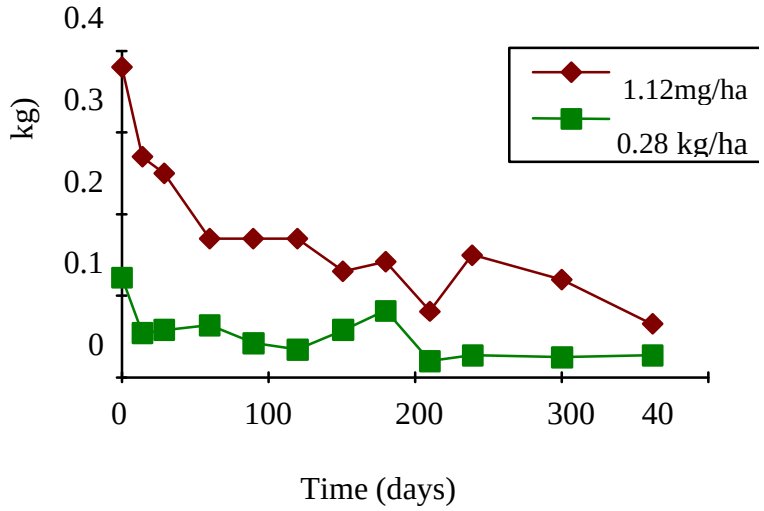
[1]Temperatures at 10 cm depth
[2]Days 240-270 only

No evaporation data were available for the Georgia site but data for the other sites indicated that the only net downward flow of water would be in the 60-120 day period in both the California trials and the 180-270 day period in the Minnesota trial (Deakyne and Stavinski, 1991)

In another study according to EPA guidelines by the same authors fenbuconazole was applied at 0.28 or 1.12 kg/ha to bare plots of 5 x 20 m in Southern California (the same location as above) on 16 May 1991, with a control plot of the same size. Five samples from

each plot taken at 0, 14, 29, 60, 90, 120, 151, 180, 210, 239, 300 and 362 days were divided into 0-15 or 0-7.5 and 7.5-15 cm depth segments and the corresponding groups of segments bulked. The samples were analysed for fenbuconazole, RH-9130, RH-9129 and RH-6467 as in the previous study. Recoveries were >88% with a limit of determination of 0.01 mg/kg.

Figure 8. Concentrations of fenbuconazole in 0-15 cm soil layers after applications of 1.12 kg/ha and 0.28 kg/ha (USA, 1991).




At both rates the half-lives of fenbuconazole were approximately 50 days and the DT90s >362 days. The concentrations in the upper layer were generally at least 20 times those in the lower layer and the highest level found in the lower layer at day 60 was approximately 7% of the applied dose.

In the upper layer at the high application rate, RH-9130 reached a peak concentration of 0.063 mg/kg after 14 days, RH-6467 0.047 mg/kg after 29 days and RH-9129 0.05 mg/kg after 90 days. All were still above the limit of detection after 300-362 days. None were detected in the lower layer at any time.

The plots received a total of 10 cm water by precipitation and irrigation during the first 160 days of the experiment, when the average soil temperature at a 20 cm depth was 29.4°C (Deakyne and Stavinski, 1993).

Sterile hydrolysis

In a study conducted according to EPA guidelines triazole-labelled fenbuconazole (0.1 µg/ml final concentration, specific activity 775 MBq/g, purity 99.1%) was incubated at 25°C in the dark in buffered solutions (10 ml) at pH 5, 7 and 9. After 0, 1, 2, 4, 8, 15, 22 and 30 days duplicate samples were extracted with ethyl acetate and analysed by TLC.

The $^{14}C$ balance averaged 99.4% during the study. Fenbuconazole was the only compound identified and accounted for >97% of the AR at all times. No degradation was detected (O'Dowd 1990a,d).

Aqueous photolysis

In a study according to EPA guidelines phenyl-labelled fenbuconazole (1.5 µg/ml final concentration, specific activity 753 MBq/g, purity 96.2%) in pH 7 phosphate buffer (40 ml) was irradiated with a xenon arc lamp (150 W/m$^2$, similar to natural sunlight in New Jersey) fitted with a filter to remove wavelengths below 290 nm. The system was maintained at 25°C and subjected to cycles of 12 h light followed by 12 h darkness. Volatile compounds were

trapped and duplicate samples were taken at 0, 3, 7, 14, 21 and 30 days, extracted with ethyl acetate and analysed by TLC or HPLC. The overall $^{14}C$ balance averaged 105% of the AR and the only compound detected was fenbuconazole (always >99% of the AR over the 30-day period). At no time was radioactivity detectable in the traps. Dark controls also showed no degradation (Wang, 1991b).

The photolysis of triazole-labelled fenbuconazole (>99% radiochemical purity, 1.5 mg/l) was also examined in natural pond water. It was moderately unstable in the presence of simulated sunlight (xenon arc lamp) at 24.1±0.6°C. Irradiation was intermittent with light and dark cycles of 12 hours each day for 30 days. Samples were taken at 0, 3, 7, 14, 21, and 30 days and analysed by solvent-solvent extraction, LSC, TLC, and HPLC with radiometric detection. Overall recoveries were high at 101-107% from the irradiated samples and 104-109% from the control samples. In addition to fenbuconazole, which accounted for 75-94% of the total applied radioactivity after 30 days of irradiation, at least eight photodegradation products were detected but none exceeded 10% of the TRR. Radioactivity in water-soluble compounds gradually increased with time, reaching about 18% of the applied radioactivity at day 30. Less than 0.1% of the applied radioactivity was volatile. The identified products included RH-6467 (2.8%), triazole (3.3%) and RH-1311 (2.7%). A mixture of polar compounds totalling 17.9% was stated to be an artifact of the co-solvent used in the sample preparation. Assuming pseudo-first-order kinetics, the half-life of fenbuconazole under the test conditions was calculated to be 86.7 days (Baur, 1994).

Sediment/water systems

In a study conducted according to BBA guidelines Rhine and pond systems containing water (550 ml, approximately 6 cm depth) and sediment (250 g, approximately 2.5 cm depth) were allowed to equilibrate for approximately 20 days. Triazole-labelled fenbuconazole (0.13 or 0.0134 mg, specific activity 835 MBq/g, purity 97.3%) was then added and traps for volatile compounds were connected to the flasks (NaOH solution to trap $CO_2$, ethylene glycol to trap organic volatiles). The flasks were incubated in the dark at 20°C while $CO_2$-free air was continuously passed through and the water phase was agitated. Duplicate samples of water and sediment were taken at 0, 7, 30, 62 and 105 days, and from the higher rate systems also at 6 h, 1, 2 and 14 days. Water samples were passed through solid-phase extraction columns for analyses by HPLC. Sediment samples were extracted with acetonitrile/acetic acid and the extracts analysed by TLC. After extraction, soil was combusted to determine the unextractable radioactivity.

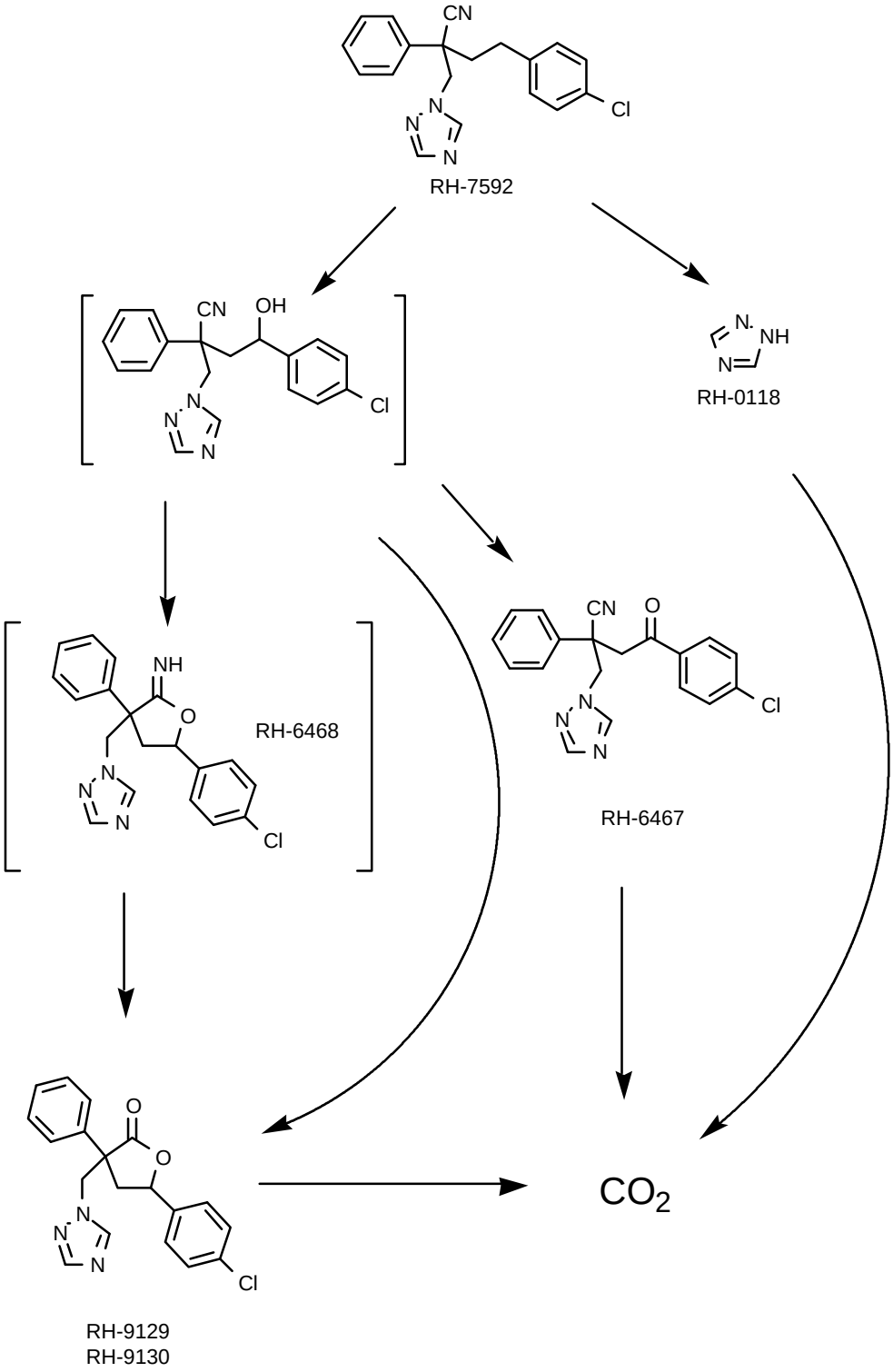
At all time intervals the total $^{14}C$ balance for both systems and both rates was >93%. There was very little difference between the results from the high and low rates and only results at the high rate are reported here. Volatile compounds accounted for 0.1% of the AR) and evolved $^{14}CO_2$ reached 0.3% after 105 days. Unextractable residues reached 6.6 and 12% of the AR after 105 days in the Rhine and pond sediments respectively. In both systems fenbuconazole was the major source of the radioactivity in the water and sediment and only very small amounts of other unidentified compounds were detected in either system (<0.7% in water, <4% in sediment). The fenbuconazole partitioned from the water to the sediment with a calculated first-order dissipation half-life from the water phase of 3.4 and 1.2 days in the Rhine and pond systems respectively and after 105 days the fenbuconazole in the sediment accounted for 79.4% of the AR in the Rhine system and 80.3% in the pond system (Volkl, 1992).

Biodegradability

In a study conducted according to OECD guidelines standard BOD bottles filled with nutrient medium (280 ml, containing inorganic salts), activated sludge bacterial inoculum and fenbuconazole (2 mg/l, 94-99.5% purity) were incubated in the dark at 20°C, together with

negative and positive controls. Duplicate bottles were analysed for dissolved oxygen at days 0, 5, 15, and 28. The chemical oxygen demand (COD) was determined by sample digestion. After 28 days the degradation was 17%. The manufacturer has proposed the degradation pathways for fenbuconazole in soil shown in Figure 9 (Costlow, 1997a; Douglas,1990).

Figure 9. Degradation pathways of fenbuconazole in soil.

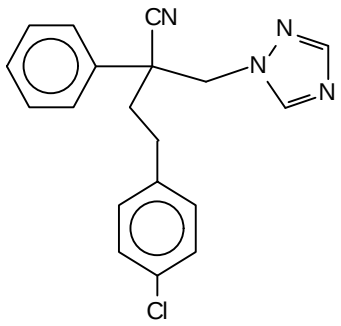
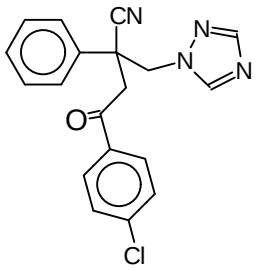
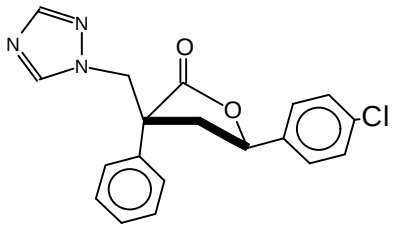
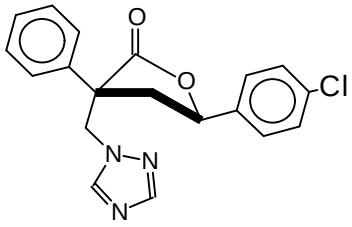
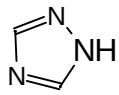
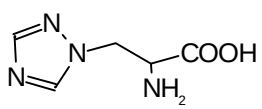


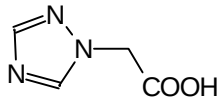
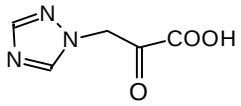
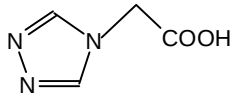
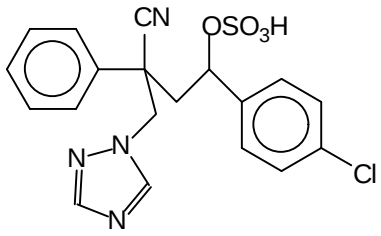
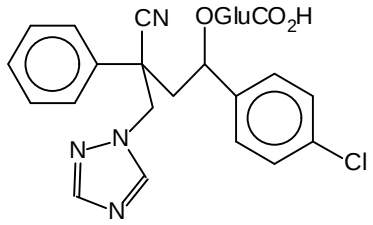
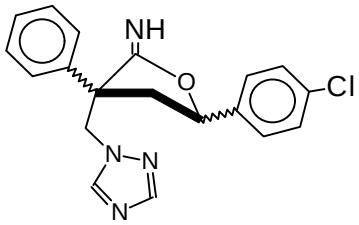
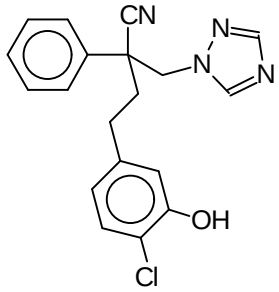
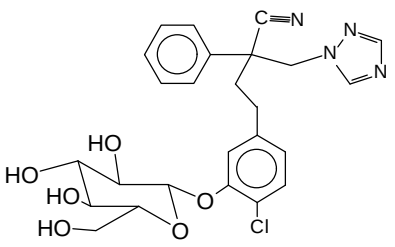

A number of studies on bioaccumulation in fish were also submitted but not reviewed (Forbis 1987; O'Dowd 1988, 1990c).

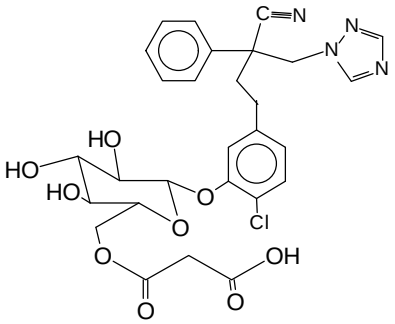
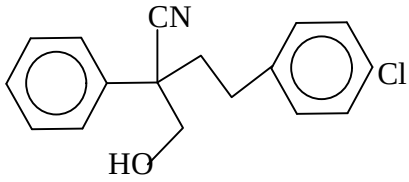
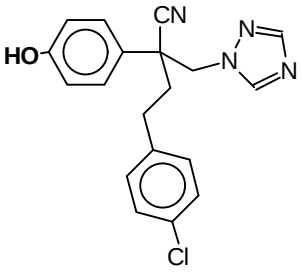
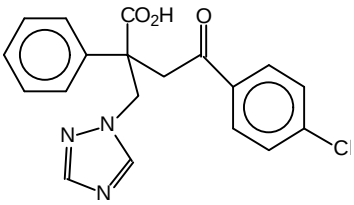
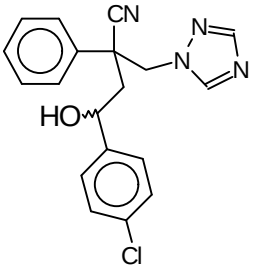
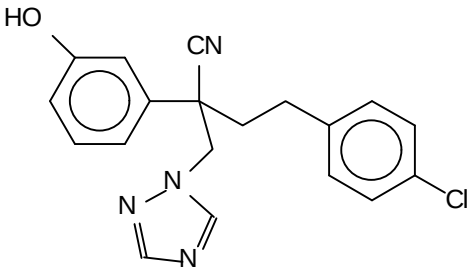
The names and structures of fenbuconazole and its degradation products and metabolites are given in Table 23.

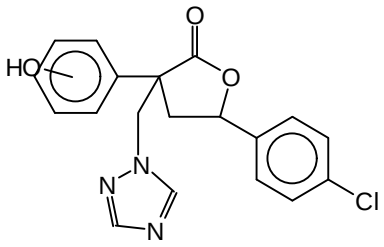
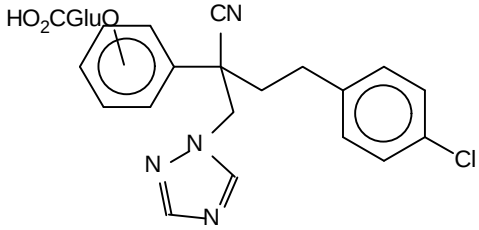
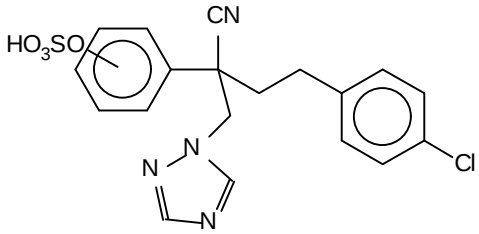
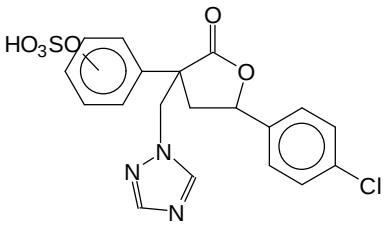
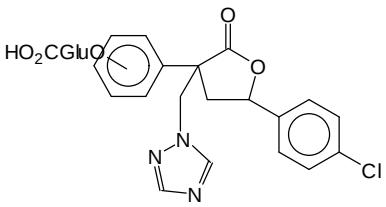
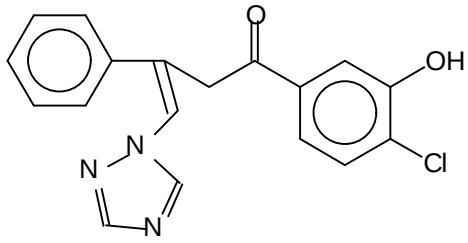
Table 23. Names and structures of fenbuconazole and its degradation products and metabolites.

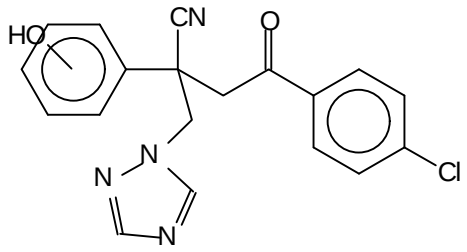
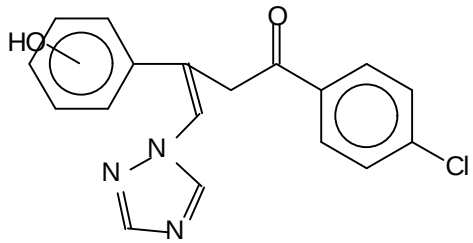
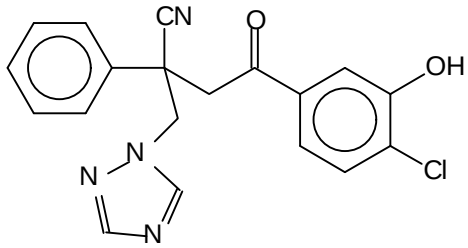
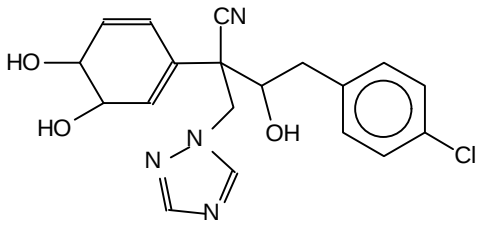
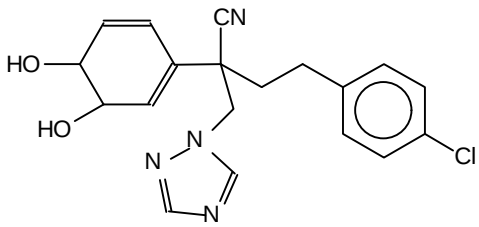
Table 23. Names and structures of fenbuconazole and its degradation products and metabolites.

| Number | Structure | Name (code name/ chemical name) | Occurrence |
|---|---|---|---|
| 1 |  | fenbuconazole (RH-7592) | Soil, wheat, peach, peanut, rotation crops, hen, goat, fish, rat |
| 2 |  | ketone (RH-6467) | Soil, wheat, peanut, rotation crops, fish, rat |
| 3 |  | lactone A (RH-9129) | Soil, wheat, peach, peanut, rotation crops, hen, goat, fish, rat |
| 4 |  | lactone B (RH-9130) | Soil, rotation crops, hen, goat, rat |
| 5 |  | triazole (RH-0118) | Soil, hen, goat, rat |
| 6 |  | triazolylalanine (RH-3968) | Wheat, peach, peanut, rotation crops, hen, goat |

| Number | Structure | Name (code name/ chemical name) | Occurrence |
|---|---|---|---|
| 7 |  | triazol-1-ylacetic acid (TAA, RH-4098) | Wheat, peach, peanut, rotation crops |
| 8 |  | triazolylpyruvic acid | Peach |
| 9 |  | triazol-4-ylacetic acid symmetrical TAA | Rotational crops |
| 10 |  | benzylic sulfates (RH-6649) | Hen, goat, fish, rat |
| 11 |  | benzylic glucuronides | Hen, goat, fish, rat |
| 12 |  | iminolactones (RH-6468) | Peanut, hen, goat, rat |
| 13 |  | 4-chloro-3-hydroxyphenyl derivative (RH-4911) | Rat |
| 14 |  | glucoside of RH-4911 | Wheat, peach, peanut |

| Number | Structure | Name (code name/ chemical name) | Occurrence |
|---|---|---|---|
| 15 |  | malonylglucoside of RH-4911 | Wheat, peach, peanut |
| 16 |  | intermediate I (RH-7968) | Hen, goat |
| 17 |  | 4-phenol (RH-1311) | Hen, goat, rat |
| 18 |  | keto acid (RH-1745) | Hen, goat, rat |
| 19 |  | benzylic alcohols (RH-6648) | Goat, rat |
| 20 |  | 3-phenol | Rat |

| Number | Structure | Name (code name/ chemical name) | Occurrence |
|---|---|---|---|
| 21 |  | phenol lactone | Rat |
| 22 |   | phenol conjugates | Rat |
| 23 |   | phenol lactone conjugates | Rat |
| 24 |  | unsaturated keto m-chlorophenol | Rat |

| Number | Structure | Name (code name/ chemical name) | Occurrence |
|---|---|---|---|
| 25 |  | keto phenol | Rat |
| 26 |  | unsaturated keto phenol | Rat |
| 27 |  | keto *m*-chlorophenol | Rat |
| 28 |  | hydroxy dihydrodiol | Rat |
| 29 |  | dihydrodiol | Rat |

## METHODS OF RESIDUE ANALYSIS

### Analytical methods

Methods have been described for the determination of fenbuconazole, the lactones, the alcohol RH-7968 and the ketone RH-6467 by GLC. The glucose conjugate of the chlorophenol RH-4911 has been determined by HPLC.

Animal products

a) Several studies were submitted, all with the same method for the determination of fenbuconazole, the lactones and 4-(4-chlorophenyl)-2-hydroxymethyl-2-phenylbutyronitrile (RH-7968) in meat, fat, milk and eggs. Samples (apparently from goats and hens, although this was not clear) were first homogenised and then extracted by blending with methanol (hexane for fat). The extract was partitioned with hexane/water (methanol/water for fat). After adding 10% sodium chloride, the aqueous layer was partitioned with dichloromethane, the dichloromethane extract evaporated to dryness and the residual material dissolved in toluene for clean-up and separation on a silica gel column. The first eluate containing RH-7968 was further cleaned up on a Florisil column, and the second fraction containing the parent compound and the lactones on a C-18 solid-phase extraction column. The separate fractions were analysed by capillary GLC with NP detection. The limit of determination was 0.01 mg/kg for fenbuconazole and the lactones and 0.05 mg/kg for RH-7968. Acceptable recoveries were demonstrated at fortification levels of 0.02-0.2 mg/kg (0.06-0.6 mg/kg for RH-7968). Recoveries were higher (124-138%) at the lowest fortification level of 0.01 mg/kg. Acceptable chromatograms were submitted (Filchner *et al.*, 1992,1994a; Haines *et al.*, 1992).

The results of validation experiments are shown in Table 24.

Table 24. Validation of residue method a) for products of animal origin.

| Substrate | Analyte | Recovery, % | Limit of determination, mg/kg |
|---|---|---|---|
| Milk | fenbuconazole | 94 (mean) | 0.01 |
| | lactones | 92 (mean) | 0.01 |
| | RH-7968 | | 0.05 |
| Muscle | fenbuconazole | 98 (mean) | 0.01 |
| | lactones | 95 (mean) | 0.01 |
| | RH-7968 | | 0.05 |
| Liver | fenbuconazole | 103 (mean) | 0.01 |
| | lactones | 102 (mean) | 0.01 |
| | RH-7968 | 79 (mean) | 0.05 |
| Kidneys | fenbuconazole | 82 (mean) | 0.01 |
| | lactones | 84 (mean) | 0.01 |
| | RH-7968 | 85 (mean) | 0.05 |
| Fat | fenbuconazole | 104 (mean) | 0.01 |
| | lactones | 107 (mean) | 0.01 |
| | RH-7968 | 106 (mean) | 0.05 |
| Egg | fenbuconazole | 95 (mean) | 0.01 |
| | lactones | 96 (mean) | 0.01 |
| | RH-7968 | | 0.05 |
| Milk | fenbuconazole | 80-114 | 0.01 |
| | RH-9130 | 74-113 | 0.01 |
| | RH-9129 | 75-116, 142 | 0.01 |
| Egg | fenbuconazole | 84-138 | 0.01 |
| | RH-9130 | 85-128 | 0.01 |
| | RH-9129 | 83-124 | 0.01 |

Some additional recoveries by method a) were reported for cow and hen tissues. Fortification levels were 0.01 to 0.05 mg/kg. The results are shown in Table 25 (Stavinski, 1994a).

Table 25. Recoveries of fenbuconazole and the lactones from cow and hen tissues. Limit of determination 0.01 mg/kg for all samples.

| Substrate | Analyte | Recovery, % Fortification level | | |
|---|---|---|---|---|
| | | 0.01 mg/kg | 0.02 mg/kg | 0.05 mg/kg |
| Cow muscle | fenbuconazole | 103-113 | 97-104 | 90-93 |
| | RH-9130 | 106-131 | 96-102 | 92-96 |
| | RH-9129 | 92-117 | 102-113 | 93-101 |
| Cow liver | fenbuconazole | 97-108 | 99-140 | 92-102 |
| | RH-9130 | 92-108 | 83-101 | 93-100 |
| | RH-9129 | 84-102 | 93-96 | 88-92 |
| Cow kidney | fenbuconazole | 83-100 | 82-110 | 81-94 |
| | RH-9130 | 89-116 | 88-113 | 85-100 |
| | RH-9129 | 93-138 | 90-114 | 98-101 |
| Cow fat | fenbuconazole | 31-99 | 50-92 | 58-90 |
| | RH-9130 | 83-117 | 55-100 | 56-116 |
| | RH-9129 | 56-114 | 65-94 | 62-116 |
| Hen muscle | fenbuconazole | 93-120 | 80-117 | 88-97 |
| | RH-9130 | 112-155 | 87-119 | 90-100 |
| | RH-9129 | 109-166 | 84-120 | 92-96 |
| Hen liver | fenbuconazole | 98-108 | 90-111 | 90-106 |
| | RH-9130 | 103-137 | 93-139 | 98-105 |
| | RH-9129 | 91-112 | 93-109 | 92-102 |
| Hen fat | fenbuconazole | 110-128 | 103-112 | 96-104 |
| | RH-9130 | 110-131 | 105-114 | 100-110 |
| | RH-9129 | 101-125 | 108-115 | 101-107 |

In a study by Sharma and Robinson (1996) samples of liver and fat from a goat metabolism study were analysed both by method a) and by TLC with radiometric detection. The results were well correlated indicating that method a) adequately extracts and quantifies the residues. The results are summarized in Table 26.

Table 26. Comparison of analyses of liver and fat by radio-TLC and analytical method a).

| Analyte | Residues, mg/kg | | | |
|---|---|---|---|---|
| | Liver | | Fat | |
| | Analyses by radio-TLC | Analyses by method a)[1] | Analyses by radio-TLC | Analyses by method a)[1] |
| | | | | |
| Fenbuconazole | 1.43 | 1.09 | 0.17 | 0.13 |
| RH-7968 | 0.05 | 0.03 | 0.04 | 0.04 |
| RH-9129 | 0.18 | 0.26 | 0.11 | 0.04 |
| RH-9130 | 0.44 | 0.24 | 0.04 | 0.02 |

| Analyte | Residues, mg/kg | | | |
|---|---|---|---|---|
| | Liver | | Fat | |
| | Analyses by radio-TLC | Analyses by method a)[1] | Analyses by radio-TLC | Analyses by method a)[1] |
| RH-6468 | 0.12 | quantified as RH-9129/RH-9130 | not detected | Quantified as RH-9129/RH-9130 |
| Sum of RH-9129, RH-9130, and RH-6468 | 0.74 | 0.50 | 0.16 | 0.05 |

[1]Means of triplicate analyses, except RH-7968

Plant commodities

Several minor variants of a basic method have been described for individual commodities. Most of the commodities in the supervised trials were analysed by a method validated for stone fruit or one for almonds.

b) This method was developed for the determination of fenbuconazole and the lactones RH-9129 and RH-9130 in stone fruit but was used for all the fruit and vegetables in the supervised trials except sugar beet. Fenbuconazole is extracted with methanol and partitioned with sodium chloride and dichloromethane. The dichloromethane is evaporated and the sample redissolved in toluene/acetone and cleaned up by silica gel and Florisil chromatography before analysis by GLC with NP detection. The linearity of response and sample chromatograms was acceptable. The limit of determination was 0.01 mg/kg. The recoveries are shown in Table 27 (Martin, 1998a, 1990, 1993a). A confirmatory method, with GLC on a different column, has been validated for stone fruit by Burnett (1991i).

Table 27. Recoveries of fenbuconazole and lactones from stone fruit.

| Sample | Fortification, mg/kg | Recovery, % | No | Reference |
|---|---|---|---|---|
| Peaches/fenbuconazole | 0.04 | 81 | 1 | Martin, 1988a |
| | 0.08 | 78 | 1 | |
| | 0.10 | 82, 114 | 2 | |
| | 0.20 | 75-101 | 4 | |
| | 0.40 | 62-116 | 4 | |
| | 0.65 | 115 | 1 | |
| | 0.80 | 94 | 1 | |
| | 1.0 | 95-103 | 3 | |
| | 1.6 | 94 | 1 | |
| Cherries/fenbuconazole | 0.10 | 110 | 1 | |
| | 0.20 | 84, 94 | 2 | |
| | 0.30 | 95-109 | 3 | |
| | 0.40 | 92 | 1 | |
| | 0.45 | 75 | 1 | |
| | 0.50 | 85, 92 | 2 | |
| Plums/fenbuconazole | 0.01 | 75-93 | 3 | |
| | 0.10 | 93-97 | 3 | |
| | 0.20 | 94 | 1 | |
| | 0.50 | 91 | 1 | |
| Cherries/fenbuconazole | 0.01 | 93, 100 | 2 | Martin, 1990, 1993a |
| | 0.04 | 91 | 1 | |
| | 0.08 | 95 | 1 | |

| Sample | Fortification, mg/kg | Recovery, % | No | Reference |
|---|---|---|---|---|
| | 0.10 | 87 | 1 | |
| | 0.40 | 84 | 1 | |
| | 2.0 | 90 | 1 | |
| | 3.0 | 78, 83 | 2 | |
| | 4.0 | 93 | 1 | |
| Cherries/RH-9130 | 0.01 | 88, 99 | 2 | |
| | 0.04 | 90 | 1 | |
| | 0.08 | 90 | 1 | |
| | 0.10 | 85 | 1 | |
| | 0.40 | 93 | 1 | |
| | 2.0 | 95 | 1 | |
| | 3.0 | 81, 83 | 2 | |
| | 4.0 | 98 | 1 | |
| Cherries/RH-9129 | 0.01 | 86, 94 | 2 | |
| | 0.04 | 77 | 1 | |
| | 0.08 | 88 | 1 | |
| | 0.10 | 74 | 1 | |
| | 0.40 | 90 | 1 | |
| | 2.0 | 87 | 1 | |
| | 3.0 | 60, 71 | 2 | |
| | 4.0 | 85 | 1 | |

c) This method has been validated for the determination of fenbuconazole and the lactones RH-9129 and RH-9130 in almonds, but also applied to fruits, vegetables, cereals and oilseed in the supervised trials. Almond hulls are Soxhlet-extracted with methanol, and kernels with toluene/methanol. The analysis is completed as above, except that clean-up on a C-18 column is included. Recoveries, linearity of response and sample chromatograms were acceptable. The limit of determination in almonds was 0.01 mg/kg for all the analytes. Recoveries are shown in Table 28 (Ross, 1996).

Table 28. Recoveries of fenbuconazole and lactones from almonds (Ross, 1996).

| Sample/analyte | Fortification, mg/kg | Recovery, % | No. |
|---|---|---|---|
| Hull/fenbuconazole | 0.01 | 86 | 1 |
| Nut/fenbuconazole | 0.01 | 82, 86 | 2 |
| Hull/fenbuconazole | 0.02 | 97, 101 | 2 |
| Kernel/fenbuconazole | 0.05 | 103 | 1 |
| Hull/fenbuconazole | 0.10 | 90-105 | 3 |
| Kernel/fenbuconazole | 0.10 | 94, 112 | 2 |
| Nut/fenbuconazole | 0.25 | 104 | 1 |
| Hull/fenbuconazole | 0.25 | 102, 107 | 2 |
| Nut/fenbuconazole | 0.5 | 99 | 1 |
| Hull/fenbuconazole | 0.5 | 96, 107 | 2 |
| Kernel/fenbuconazole | 1.0 | 103 | 1 |
| Hull/fenbuconazole | 1.0 | 72 | 1 |
| Hull/RH-9129 | 0.01 | 125 | 1 |
| Nut/ RH-9129 | 0.01 | 75 | 1 |
| Hull/ RH-9129 | 0.02 | 97, 98 | 2 |

| Sample/analyte | Fortification, mg/kg | Recovery, % | No. |
|---|---|---|---|
| Kernel/ RH-9129 | 0.05 | 103 | 1 |
| Hull/ RH-9129 | 0.10 | 93-107 | 3 |
| Kernel/ RH-9129 | 0.10 | 105, 115 | 2 |
| Hull/ RH-9129 | 0.25 | 105, 110 | 2 |
| Kernel/ RH-9129 | 0.5 | 97 | 1 |
| Hull/ RH-9129 | 0.5 | 99, 108 | 2 |
| Kernel/ RH-9129 | 1.0 | 107 | 1 |
| Hull/ RH-9129 | 1.0 | 78 | 1 |
| Hull/RH-9130 | 0.01 | 107 | 1 |
| Nut/ RH-9130 | 0.01 | 77, 83 | 2 |
| Hull/ RH-9130 | 0.02 | 76, 97 | 2 |
| Kernel/ RH-9130 | 0.05 | 108 | 1 |
| Hull/ RH-9130 | 0.10 | 97-102 | 3 |
| Kernel/ RH-9130 | 0.10 | 93, 105 | 2 |
| Nut/ RH-9130 | 0.25 | 105 | 1 |
| Hull/ RH-9130 | 0.25 | 100, 109 | 2 |
| Nut/ RH-9130 | 0.5 | 99 | 1 |
| Hull/ RH-9130 | 0.5 | 93, 109 | 2 |
| Kernel/ RH-9130 | 0.5 | 93 | 1 |
| Kernel/ RH-9130 | 1.0 | 96 | 1 |
| Hull/ RH-9130 | 1.0 | 72 | 1 |

The same method (Ross, 1996) has been validated for the determination of fenbuconazole, the lactones, and the ketone RH-6467 in wheat. It was also applied to sugar beet and oilseed in the supervised trials. The limit of determination for all the analytes was 0.05 mg/kg in straw and 0.01 mg/kg in grain. The recoveries are shown in Table 29. The identities of the analytes were confirmed by GLC on a different column (Burnett *et al.*, 1994a,b; Martin, 1991c).

Table 29. Recoveries of fenbuconazole, RH-9129, RH-9130 and RH-6467 from wheat (Burnett *et al.*, 1994a,b; Martin, 1991c).

| Sample/analyte | Fortification, mg/kg | Recovery-% | No. | Reference |
|---|---|---|---|---|
| Grain/fenbuconazole | 0.01 | 77-106 | 3 | Burnett *et al.*, 1994a |
| | 0.02 | 63-101 | 4 | |
| | 0.03 | 69-81 | 3 | |
| | 0.04 | 78 | 1 | |
| | 0.05 | 68-104 | 6 | |
| | 0.10 | 90-106 | 6 | |
| Grain/RH-9130 | 0.01 | 92-101 | 3 | |
| | 0.02 | 68-110 | 4 | |
| | 0.03 | 74-83 | 3 | |
| | 0.04 | 81 | 1 | |
| | 0.05 | 70-106 | 6 | |
| | 0.10 | 92-109 | 6 | |
| Grain/RH-9129 | 0.01 | 75-96 | 3 | |
| | 0.02 | 63-103 | 4 | |
| | 0.03 | 69-73 | 3 | |

| Sample/analyte | Fortification, mg/kg | Recovery-% | No. | Reference |
|---|---|---|---|---|
| | 0.04 | 72 | 1 | |
| | 0.05 | 66-106 | 6 | |
| | 0.10 | 80-108 | 6 | |
| Grain/RH-6467 | 0.01 | 79-111 | 3 | |
| | 0.02 | 73-118 | 4 | |
| | 0.03 | 80-94 | 3 | |
| | 0.04 | 91 | 1 | |
| | 0.05 | 72-112 | 6 | |
| | 0.10 | 83-117 | 6 | |
| Straw/fenbuconazole | 0.05 | 80-91 | 6 | |
| | 0.10 | 66-109 | 3 | |
| | 0.20 | 70-93 | 4 | |
| | 0.50 | 82-109 | 7 | |
| | 1.0 | 79-99 | 4 | |
| | 2.0 | 89-113 | 5 | |
| Straw/RH-9130 | 0.05 | 79-92 | 6 | |
| | 0.10 | 67-101 | 3 | |
| | 0.20 | 82-102 | 4 | |
| | 0.50 | 76-109 | 7 | |
| | 1.0 | 79-90 | 4 | |
| | 2.0 | 89-113 | 5 | |
| Straw/RH-9129 | 0.05 | 79-96 | 6 | |
| | 0.10 | 69-100 | 3 | |
| | 0.20 | 85-95 | 4 | |
| | 0.50 | 77-123 | 7 | |
| | 1.0 | 75-92 | 4 | |
| | 2.0 | 93-114 | 5 | |
| Straw/RH-6467 | 0.05 | 81-94 | 6 | |
| | 0.10 | 62-112 | 3 | |
| | 0.20 | 78-115 | 4 | |
| | 0.50 | 78-114 | 7 | |
| | 1.0 | 79-92 | 4 | |
| | 2.0 | 89-122 | 5 | |
| Straw/fenbuconazole | 1.0 | 78-100 | 4 | Burnett *et al.*, 1994b |
| | 5.0 | 88-99 | 3 | |
| | 10.0 | 80-108 | 6 | |
| | 15.0 | 79-114 | 6 | |
| Straw/RH-9130 | 1.0 | 79-97 | 4 | |
| | 5.0 | 92-98 | 3 | |
| | 10.0 | 78-107 | 6 | |
| | 15.0 | 77-114 | 6 | |
| Straw/RH-9129 | 1.0 | 81-100 | 4 | |
| | 5.0 | 79-93 | 3 | |
| | 10.0 | 74-109 | 6 | |
| | 15.0 | 81-114 | 6 | |
| Straw/RH-6467 | 1.0 | 75-93 | 4 | |
| | 5.0 | 82-88 | 3 | |
| | 10.0 | 56-94 | 6 | |

| Sample/analyte | Fortification, mg/kg | Recovery-% | No. | Reference |
|---|---|---|---|---|
| | 15.0 | 60-106 | 6 | |

d) A method developed for the determination of the same compounds in pecans was identical except that samples were Soxhlet-extracted with hexane/2-propanol and concentrated to an oily residue before dissolution in toluene/acetone. The limit of determination was 0.01 mg/kg for all the analytes with the recoveries shown in Table 30 (Martin, 1991a,b; Wu, 1994). A confirmatory procedure, with GLC on a different column has been validated (Burnett, 1991i).

Table 30. Recoveries of fenbuconazole, the lactones and the ketone from pecans.

| Compound | Fortification, mg/kg | Mean (±sd) | Recovery,-% | No. |
|---|---|---|---|---|
| fenbuconazole | 0.01 | 85 (21) | 49-104 | 7 |
| | 0.02 | 98 (8) | 84-108 | 6 |
| | 0.04 | 92 (19) | 70-122 | 8 |
| RH-9130 | 0.01 | 91 (26) | 55-123 | 7 |
| | 0.02 | 101 (14) | 75-113 | 6 |
| | 0.04 | 91 (17) | 67-114 | 8 |
| RH-9129 | 0.01 | 89 (27) | 51-122 | 7 |
| | 0.02 | 91 (18) | 67-112 | 6 |
| | 0.04 | 79 (14) | 64-99 | 8 |
| RH-6467 | 0.01 | 100 (33) | 84-153 | 7 |
| | 0.02 | 105 (25) | 71-134 | 6 |
| | 0.04 | 93 (15) | 61-111 | 8 |

e) The same method, but with GLC on a capillary column, has been used for the determination of fenbuconazole, the ketone and the lactones in wheat, and fenbuconazole and the lactones in apples. The limit of determination was 0.01 mg/kg for wheat grain and 0.05 mg/kg for straw and apples. Acceptable chromatograms were submitted. The recoveries are shown in Table 31 (Burnett *et al.*, 1992a,b; Martin, 1989).

Table 31. Recoveries of fenbuconazole and metabolites from wheat and apples.

| Sample | Fortification, mg/kg | Recovery, % | | |
|---|---|---|---|---|
| | | fenbuconazole | ketone | lactones |
| Wheat grain | 0.02-1.0 | 63-111<br>mean 91 | 72-118 | 63-110<br>mean 89 |
| Straw | 0.05-5.0 | 67-118<br>mean 94 | 62-122<br>mean 91 | 54-123<br>mean 89 |
| Apples | 0.1-1.0 | 71-114 | - | 65-109<br>mean 84 |

f) A somewhat simpler method has been used for the determination of fenbuconazole and the lactones RH-9129 and RH-9130 in wheat and grapes. Samples were extracted with acetone/petroleum ether or hexane, cleaned up on a Florisil column, and analysed by GLC with an FID. The limit of determination was stated to be 0.01 mg/kg. Recoveries from wheat grain and grapes fortified at 0.25 mg/kg were 92 ± 8%. Recoveries from grapes fortified with

fenbuconazole at 0.1 to 0.25 and 1 mg/kg and “its lactone” at 0.2 to 0.4 and 2 mg/kg were about 100 ± 10%. A satisfactory sample chromatogram was submitted (Mestres, 1989).

g) A preliminary method for the determination of RH-7905, the glucose conjugate of the phenol RH-4911, in wheat grain, bananas and apples has been described. The conjugate was hydrolysed to its phenol with acid in methanol and the hydrolysate partitioned with sodium chloride solution and dichloromethane. The dichloromethane was evaporated and the residue redissolved in acetonitrile before LC-18 SPE clean-up and analysis by HPLC-MS. Satisfactory sample chromatograms were provided. The average recoveries were 87% (sd ± 2.5) from wheat grain, 84% (sd ± 1.9) from apples, and 90% (sd ± 7.3) from bananas. Only summary information was provided on recoveries; the report states that the limit of determination will be determined in further experiments (Staurowsky and Novak, 1996).

The methods of analysis used in the residue trials on the individual commodities are listed in Table 32.

Table 32. Summary of the methods of analysis used in supervised trials (Ross, 1997a).

| Commodity | Method | | |
|---|---|---|---|
| | b) | d) | c) |
| Citrus fruits | | | |
| Grapefruit | yes | | |
| Orange | yes | | |
| Pome fruits | | | |
| Apple | yes | | yes |
| Loquat | yes | | |
| Pear | yes | | yes |
| Stone fruits | | | |
| Apricot | yes | | |
| Cherries | yes | | |
| Nectarine | yes | | |
| Peach | yes | | |
| Plum / prune | yes | | yes |
| Berries | | | |
| Grape | yes | | yes |
| Strawberry | yes | | yes |
| Fruit, inedible peel | | | |
| Banana | yes | | |
| Mango | yes | | |
| Fruiting veg., Cucurbits | | | |
| Melons not watermelon | yes | | yes |
| Cucumber | yes | | yes |
| Squash, summer | yes | | |
| Fruiting veg., other than cucurbit | | | |
| Peppers | yes | | yes |
| Tomato | yes | | yes |
| Root and tuber vegetables | | | |
| Sugar beet | | | yes |
| Cereal grains | | | |
| Barley | | | yes |

| | Method | | |
|---|---|---|---|
| Maize (corn) | | | yes |
| Rye | | | yes |
| Wheat | | | yes |
| Tree nuts | | | |
| Almond | | | yes |
| Pecan | | yes | |
| Oilseeds | | | |
| Rape seed | | | yes |
| Sunflower seed | | | yes |

**Stability of pesticide residues in stored analytical samples**

Apples, wheat grain and straw. Samples were fortified at 0.5 mg/kg with fenbuconazole and the lactone metabolites, and wheat grain and straw also with the ketone, and stored in a freezer at -10°C for 36 months. Samples taken at intervals were analysed by method c). Uncorrected recoveries are shown in Tables 33-35. They show that all the analytes were stable for 36 months in wheat and apples. Procedural recoveries generally varied between 60 and 120% being somewhat higher for apples (Batra, 1995c).

Table 33. Stability of residues in fortified wheat grain stored at -10°C.

| Storage period, months | Recoveries, % | | |
|---|---|---|---|
| | Fenbuconazole | Lactone | Ketone |
| 0 | 74.9 | 73.5 | 76.7 |
| 3 | 66.3 | 66.4 | 82.9 |
| 6 | 80.0 | 77.9 | 81.1 |
| 12 | 74.9 | 69.0 | 86.4 |
| 18 | 79.0 | 81.1 | 100 |
| 24 | 94.9 | 92.4 | 90.7 |
| 30 | 98.3 | 98.1 | 119 |
| 36 | 90.4 | 86.2 | 90.3 |

Table 34. Stability of residues in fortified wheat straw stored at -10°C.

| Storage period, months | Recoveries, % | | |
|---|---|---|---|
| | Fenbuconazole | Lactone | Ketone |
| 0 | 70.3 | 61.8 | 71.0 |
| 3 | 93.0 | 94.6 | 100 |
| 6 | 83.0 | 87.4 | 97.8 |
| 12 | 80.7 | 86.6 | 96.8 |
| 18 | 103 | 101 | 113 |
| 24 | 79.9 | 77.1 | 71.8 |
| 30 | 80.0 | 79.1 | 94.7 |
| 36 | 93.7 | 95.6 | 98.7 |

Table 35. Stability of residues in fortified apples stored at -10°C.

| Storage period, months | Recoveries, % | |
|---|---|---|
| | Fenbuconazole | Lactone |
| 0 | 110 | 111 |
| 3 | 94.1 | 93.6 |
| 6 | 81.7 | 80.4 |
| 12 | 79.1 | 74.8 |
| 14 | 94.2 | 87.9 |
| 18 | 84.7 | 78.2 |
| 24 | 86.7 | 82.7 |
| 30 | 78.0 | 73.1 |
| 36 | 102 | 96.0 |

Another study on the stability of residues in frozen wheat grain, bran and shorts/germ showed that residues of fenbuconazole were stable in these substrates for up to 29 months (Burnett, 1992g).

Wheat grain, bran, middlings, shorts, red dog, low grade flour, patent flour and bread were analysed by, the method of analysis of Burnett (1994a) before and after frozen storage for approximately 4.6 years. Residues did not decrease significantly in cleaned grain, bran, shorts/germ, and red dog, but losses were substantial in middlings, flour and bread. Recoveries of 70 to 125% were reported (Burnett, 1994c).

Table 36. Stability of total residues of fenbuconazole and its lactone and ketone metabolites in processed fractions of wheat stored at -10°C for approximately 4.6 years.

| Sample | Residues, mg/kg | |
|---|---|---|
| | Before storage | After storage |
| Cleaned grain | 0.094 | 0.077 (-18%) |
| Bran | 0.536 | 0.611 (+14%) |
| Middlings | 0.108 | 0.074 (-31%) |
| Shorts/germ | 0.270 | 0.262 (-3%) |
| Red dog | 0.125 | 0.112 (-10%) |
| Flour (low grade) | 0.066 | 0.042 (-36%) |
| Patent flour | 0.104 | 0.045 (-57%) |
| Bread | 0.110 | 0.072 (-34%) |

Stone fruit. Peaches were fortified with fenbuconazole and its lactone metabolites at 0.5 mg/kg and stored in a freezer at -10°C for 54 months. Samples taken at intervals were analysed by the method of Martin (1990). Uncorrected recoveries are recorded in Table 37 and show that fenbuconazole and the lactones were stable for 54 months. Procedural recoveries of 65-120% were generally acceptable. CLICK HERE to continue