TEBUFENOZIDE

IDENTITY

ISO common name: tebufenozide

Chemical name

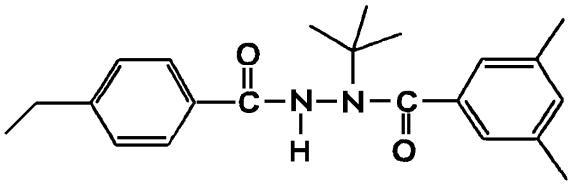
IUPAC:
N-*tert*-butyl-*N*′-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide

CA:
3,5-dimethylbenzoic acid 1-(1,1-dimethylethyl)-2-(4-ethylbenzoyl)hydrazide

CAS No: 112410-23-8

Synonyms: RH-75992, HOE-105540, MIMIC

Structural formula:



Molecular formula: $C_{22}H_{28}N_2O_2$

Molecular weight: 352

Physical and chemical properties

Pure active ingredient (Kelly, 1992b)

Vapour pressure: 2×10^{-8} torr (3×10^{-6} Pa)

Melting point: 191-191.5°C

Octanol/water partition coefficient:	mean K_{ow} 17,906. log K_{ow} 4.25
Solubility:	0.83 mg/l in water 6.5 g/100 ml in acetone 10.8 g/100 ml in methanol 24 g/100 ml in methylene chloride
bulk density:	0.28 g/ml
Hydrolysis:	stable at pH 5, 7, or 9.
Photolysis:	degraded photolytically in pond water with a half-life of 67 days (Reynolds, 1992b). Degraded photolytically when adsorbed on soil with a half-life of 98 days (Reynolds, 1991).

Technical material (Kelly, 1992a)

Purity:	94-97% (average 96%)
Melting point:	188.5-190.0°C
Stability:	stable at ambient temperatures for at least two years

Formulations

The main formulations are two aqueous suspension concentrates and a wettable powder. The suspension concentrates are the 2 SC containing 240 g ai/l and the 200 SC containing 200 g ai/l. The wettable powder contains 700 g ai/kg. Several other formulations have been developed for use specifically in rice: a 7.5 g ai/kg wettable powder, an 8 g ai/kg wettable powder, a 100 g ai/kg wettable powder, and a 1 g ai/kg granule.

METABOLISM AND ENVIRONMENTAL FATE

Radiolabels

Three different ^{14}C compounds were used. One labelled in the ring carbons of the 4-ethylphenyl ring, referred to as "A-ring labelled", the second labelled in the ring atoms of the 3,5-dimethylphenyl ring and referred to as "B-ring labelled" and the third with the central carbon of the *tert*-butyl group lebelled and referred to as "t-butyl labelled".

In studies where it was possible or likely that the entire molecule would be metabolized all three labelled compounds were used to allow for the isolation and identification of fragments of the parent compound. In other studies only one label was used.

Animal Metabolism

The metabolism of tebufenozide has been studied in rats, lactating goats, and laying hens. In each case the test material was administered orally. All the studies included test material labelled in all three positions.

Rats. The metabolism of tebufenozide in male and female rats was studied using a single high dose (250 mg/kg), a single low dose (3 mg/kg), or a pulse dose (3 mg/kg) given after dosing for two weeks with unlabelled test material. The low-dose study was with all three radiolabels, the high-dose study with the B-ring and t-butyl labels and the pulse dose study with only the t-butyl label. Each test group consisted of 5 male or 5 female rats. Pooled samples of urine and faeces from each group, each containing >95% of the excreted radioactivity, were extracted and analysed (Hawkins *et al.*, 1992b).

For the determination of metabolites samples from the 5 animals in each test group were combined for analysis. After dosing, urine and faeces were collected at approximately 24-hour intervals for 7 days.

Almost all the radioactivity (94-104%) in all test groups was eliminated in the faeces. Low levels (0.5-8.0%) were excreted in the urine and only traces of radioactivity were left in the carcase, blood and tissues or excreted as carbon dioxide or other volatile compounds.

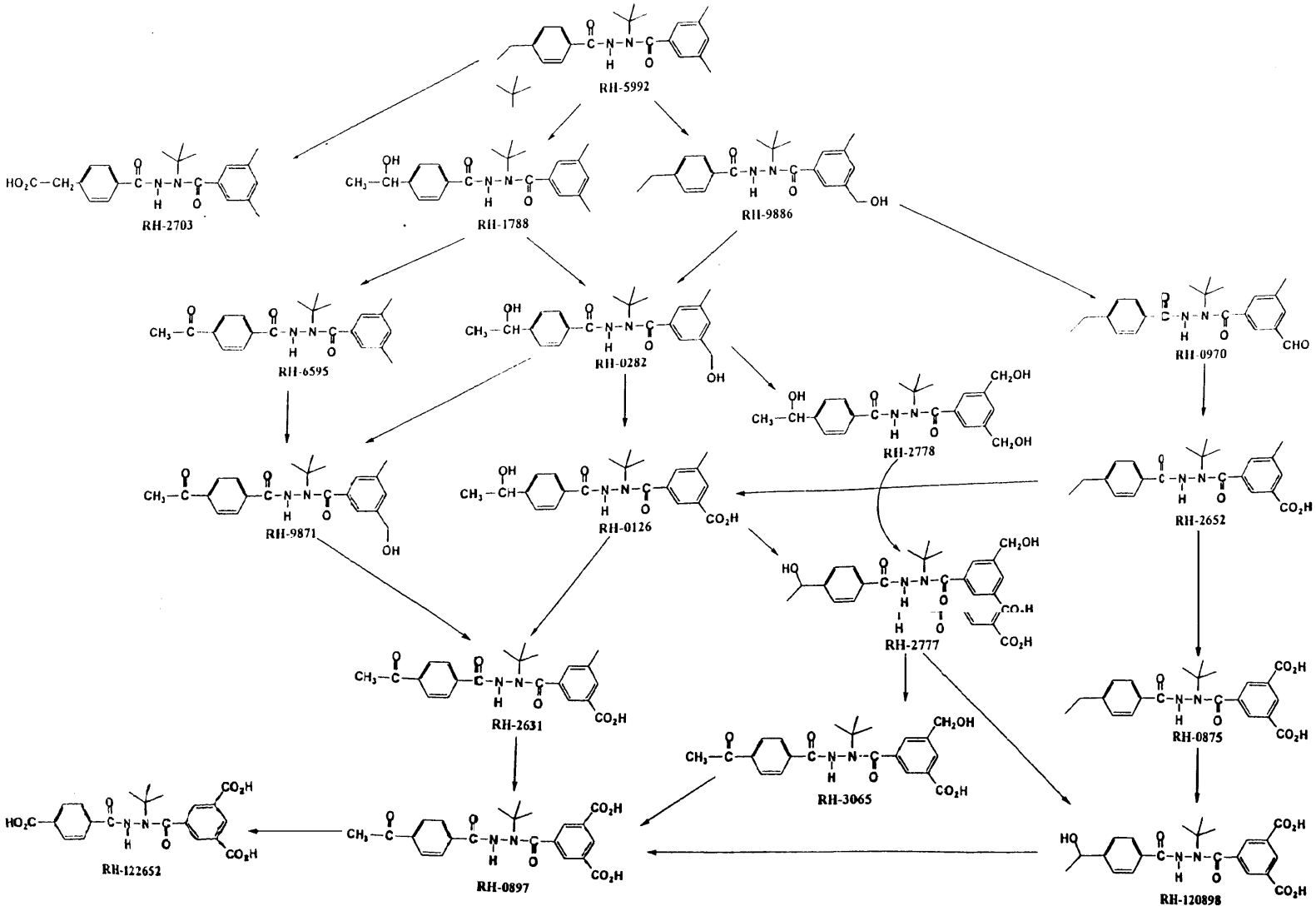
The extent of metabolism in the high- and low-dose groups was found to be highly dependent on the dose. When fed a nominal dose of 250 mg/kg, <4% of the dose was metabolized. When fed a nominal dose of 3 mg/kg about 46% was metabolized.

No qualitative differences in metabolism between the different labelled compounds were seen in the faeces samples from either high or low doses where multiple labels were used. Most of the activity in the high-dose faeces was due to parent tebufenozide. In the low-dose samples the parent accounted for 35-43% of the dosed activity. In addition to the parent compound a total of eleven metabolites were identified in the high-dose faeces samples: RH-6595, RH-1788, RH-9886, RH-2631, RH-9871, RH-0126, RH-0282, RH-0897, RH-120898, RH-0875, and RH-0970. All of these metabolites except RH-0970 were also found in the low-dose faeces samples together with four others, RH-2703, RH-3065, RH-2777, and RH-2778, which were not identified in the high-dose samples. The structures of the metabolites are shown in Figure 1.

In the pulse dose study, male and female rats were dosed with 30 ppm of unlabelled compound in the diet for two weeks before administration of a single 3 mg/kg dose of labelled tebufenozide. The metabolism in these rats was qualitatively the same as in the rats receiving only a low single dose of the test material, but higher levels of the more highly oxidized metabolites were observed (Hawkins *et al.*, 1993).

The faeces samples showed a similar metabolic profile to the low-dose samples, but they did not contain RH-9871 or RH-2703 and contained one additional metabolite, RH-122652. The parent compound accounted for 26-39% of the dose.

Figure 1. Proposed metabolic pathways of tebufenozide in rats.



No tebufenozide was found in the urine nor were several of the less polar faecal metabolites, but eleven of the metabolites identified in the faeces were also found in the urine, together with one additional whole-molecule metabolite, RH-2652. Although no differences were observed between the metabolites in the faeces produced from the three different labelled compounds the same was not true of the metabolites in the urine samples; small amounts of cleavage products containing only the A-ring or only the B-ring label (none greater than 1.5% of the dosed activity) were also seen but not identified. Some compounds with the B-ring label were similar in chromatographic behaviour to some of the whole-molecule metabolites.

As can be seen from Figure 1, all identified metabolites result from oxidation of the alkyl substituents of the aromatic rings of tebufenozide, particularly at the carbons adjacent to the rings. On the A-ring side of the molecule, this can result in either a secondary alcohol or a ketone, or an acid produced by oxidation of the terminal carbon of the ethyl group. On the B-ring side there are two positions which can be oxidized to combinations of alcohols acids.

Table 1. Tebufenozide and its metabolites in the combined urine and faeces of rats (Hawkins *et al.*, 1992, 1993).

Compound (see Figure 1)	% of ^{14}C in dose					
	High-dose[1]		Low-dose[1]		Pulse dose[2]	
	Male	Female	Male	Female	Male	Female
Tebufenozide	96.6	99.7	43.5	34.6	39.3	26.1
RH-6595	0.48	0.40	2.5	2.8	0.6	0.8
RH-2652	0.002	0.12				
RH-1788	0.19	0.31	0.58	0.8	1.7	2.0
RH-2631	0.11	0.1	10.0	1.0	3.5	3.8
RH-9886	0.19	0.28	1.04	0.99	0.2	0.26
RH-2703			0.26		0.44	0.18
RH-0126	0.22	0.03	3.83	3.3	5.7	4.5
RH-0282	1.4	1.1	2.64	13.9	11.1	16.3
RH-0875	0.06	0.11	3.8	0.1	0.57	
RH-9871	0.14	0.38	1.74	1.54	0.84	1.23
RH-0897	0.07	0.03	1.81	0.12	3.35	0.34
RH-120898	0.04		2.2	3.1	6.1	15.2
RH-2778		0.003	0.11	8.3	3.5	2.7
RH-0970	0.4	0.5				
RH-2777	0.006		0.41	0.34	0.39	10.9
RH-3065	0.02		1.63	1.05	2.49	0.41
RH-122652					1.24	4.41
Unknowns[3]	0.19	0.14	1.1	6.6	3.0	4.7
TOTAL	100.1	103.2	77.1	78.5	84.0	93.8

[1] Hawkins *et al.*, 1992
[2] Hawkins *et al.*, 1993
[3] Only whole-molecule compounds

Livestock. The fate of tebufenozide fed to lactating dairy goats was studied by dosing three goats orally with tebufenozide labelled in all three parts of the molecule at the equivalent of approximately 50 ppm in the feed for 7 consecutive days. The goats weighed between 45 and 60 kg. Milk, urine, and faeces were collected during the dosing period and the animals were killed within 24 hours after the last dose. No adverse effects of the dosing were observed. The samples collected for analysis were composite fat, consisting of equal portions of omental and perirenal fat, kidneys, liver, and composite muscle consisting of equal portions of longissimus dorsi, semimembranosus, and triceps muscle (Bender *et al.*, 1995). The total radioactivity recovered in the excreta, tissues, and milk was 88.2% of the A-ring dose, 87.3% of the B-ring, and 90.0% of the t-butyl. Table 2 shows the recovery of the administered activity in the separate samples.

Table 2. Recovery of ingested radioactivity from goats fed with [^{14}C]tebufenozide (Bender *et al.*, 1995).

Sample	% of total dose of ^{14}C		
	A-ring ^{14}C	B-ring ^{14}C	t-butyl ^{14}C
Faeces	79.0	78.3	81.1
Urine	8.9	8.6	7.8
Liver	0.07	0.12	0.4
Composite fat	0.14	0.15	0.26
Composite milk	0.09	0.08	0.26
Composite muscle	0.02	0.06	0.16
Kidney	<0.01	<0.01	0.01
Heart	<0.01	<0.01	<0.01
Total	88.2	87.3	90.0

The test compound was eliminated mainly in the faeces where 79.0%, 78.3% and 81.1% of the total dose was recovered from the A-ring, B-ring, and t-butyl labels respectively. The urine accounted for 8.9% of the A-ring, 8.6% of the B-ring, and 7.8% of the t-butyl activity. Thus 87% to 89% of the administered dose was eliminated from the body via the excreta. Less than 0.3% of the dose was excreted in the milk during the 7-day dosing period: 0.09%, 0.08%, and 0.26% of the total dose from the A-ring, B-ring, and t-butyl labels respectively. Body tissues contained small amounts of activity: fat contained the highest percentage of the dose in the A-ring and B-ring samples, 0.14% and 0.15%, and the liver the highest amount from the t-butyl label (0.4% of the dose). Detectable residues were found in the muscle, heart, and kidney of all the goats. Residue levels in the tissues expressed as tebufenozide equivalents are shown in Table 3.

Table 3. Residues of ^{14}C as tebufenozide equivalents in the milk and tissues of goats (Bender *et al.*, 1995).

Sample	^{14}C, mg/kg tebufenozide equivalents		
	A-ring ^{14}C	B-ring ^{14}C	t-butyl ^{14}C
Liver	0.50	0.99	2.87
Fat	0.17	0.14	0.29
Kidney	0.04	0.06	0.30
Muscle	0.007	0.02	0.06
Milk (day 2)	0.07	0.07	0.16

Milk, fat, liver, kidney, muscle, and urine samples were analysed to determine the nature of the residue. Milk samples were extracted with chloroform, methanol and water, fat samples with hexane and methanol, and muscle, kidney and liver with various solvent mixtures which included acetonitrile, water, chloroform and methanol. Urine samples were extracted with ethyl acetate and butanol. The quantitative determination of the metabolites was carried out by TLC, GLC, HPLC and MS.

Residue levels of ^{14}C as tebufenozide in the milk remained relatively constant throughout the dosing period. With the exception of a single residue of 0.12 mg/kg seen on day 6 from the B-ring label, residues in the A- and B-ring milk samples remained consistently in the range 0.05-0.07 mg/kg throughout the 7 days. Residues in the t-butyl milk were approximately twice those found in the other samples, from 0.09 mg/kg on day 1 to a maximum of 0.17 mg/kg on day 5.

The total radioactive residue (TRR) in the milk samples from day 2 corresponded to 0.07 mg/kg from the A- and B-ring labels and 0.16 mg/kg from the t-butyl label. More than 90% of the A- and B-ring activity was extractable with, or partitioned into, organic solvents. Only slightly more than 40% of the original t-butyl activity could be extracted in the same manner: most of the remainder, 37% of the TRR, remained in the aqueous fraction and was characterized as arising from polar, non-volatile small molecules, probably lactose and/or amino acids. The t-butyl label also had the highest proportion of unextractable activity, nearly 10%. Hydrolysis with dilute acid or base released about one third of this activity, all of which was insoluble in ethyl acetate.

In the milk, all 3 labels appeared in the same residues, generally in similar concentrations. The milk contained two major components of the TRR. One or more fatty acid conjugates of the B-ring alcohol RH-9886 represented 17-24% of the total activity from the A- and B-ring labels (0.01-0.017 mg/kg). Tebufenozide was detected at a concentration of approximately 0.01 mg/kg from each of the labels, and represented 13.7% of the TRR. Three other alcohol metabolites were also identified: RH-0282, RH-9871 and RH-9886.

Residue levels of ^{14}C as tebufenozide in fat samples ranged from 0.143 mg/kg from the B-ring label to 0.286 mg/kg from the t-butyl. More than 90% of the activity in the A- and B-ring labelled fat samples was extractable with or partitioned into organic solvents, but only about 55% of the t-butyl activity was in these fractions. Almost 18% of the t-butyl activity was found in the aqueous fraction, with close to 27% of the activity remaining in the post-extraction solids (PES). After saponification, most of the PES activity was released and partitioned into both aqueous and organic extracts. Radioactive residues in the fat comprised solely the parent compound and three fatty acid conjugates of RH-9886, the conjugated alcohol present in milk. The fatty acids were identified by mass spectrometry

as palmitic, oleic, and stearic. The relative amounts of the parent and the conjugates varied in the three samples. Residues of tebufenozide were highest in the B-ring labelled fat, contributing 69.1% of the total residue at a concentration of 0.1 mg/kg. The lowest concentration of the parent, <0.02 mg/kg, was found with the t-butyl label, where it represented only 5.4% of the TRR.

The liver contained the highest concentration of residues with all 3 labels of any of the tissues analysed, ranging from 0.5 mg/kg from the A-ring to 2.9 mg/kg from the t-butyl label. The 3-6-fold higher residue levels in the t-butyl sample suggested some fragmentation of the parent molecule.

The major component of the A- and B-ring labelled liver was RH-2703, in which the terminal group of the ethyl substituent on the A-ring has been oxidized to carboxyl. This compound was also present in large quantities in the t-butyl labelled liver, but that also showed an approximately equal amount of one of the fatty acid conjugates of RH-9886. Numerous oxidative degradation products were present with all 3 labels. The major metabolites identified with the t-butyl label were volatile: 2-propanol and acetaldehyde. No residues of the parent were seen in the liver.

Residues in kidney samples were identified as a small amount of unmetabolized parent, together with several of its metabolites. Residues of tebufenozide were well below 0.01 mg/kg with each of the labels and represented between 0.9 and 12.8% of the TRR. Residues of RH-9886, RH-0282, and RH-2631 were also identified. The highest concentration of any single metabolite was 0.015 mg/kg of RH-0282 in the B-ring labelled sample (23.3% of the TRR in the kidney).

Muscle contained the lowest levels of radioactive residues of any samples analysed. The TRR ranged from less than 0.01 mg/kg with the A-ring label to 0.06 mg/kg with the t-butyl label. The parent and 3 of its metabolites were identified with all three labels. Samples with the B-ring label contained the highest amount of unmetabolized parent, 45.1% of the TRR, at a concentration of 0.008 mg/kg. The concentrations of tebufenozide with the A-ring and t-butyl labels were 0.002 and 0.005 mg/kg respectively (32.6% and 8.9% of the TRR). Three alcohol metabolites were detected: RH-9886, RH-0282, and RH-2778. All were present at concentrations below 0.004 mg/kg. The distribution of the identified residues is shown in Table 4.

Table 4. Residues identified in goat milk and tissues (Bender *et al*, 1995).

Compound	% of total ^{14}C in sample[1]				
	Milk (day 2)	Fat	Liver	Kidney	Muscle
Tebufenozide	13.7	47.4	-	11.4	38.9
Fatty acid conjugates(s) of RH-9886	20.8	20.5	5.0	-	-
RH-0282	9.3	-	2.7	15.7	24.8
RH-9871	6.7	-	1.2	-	-
RH-9886	1.0	-	2.4	12.1	10.1
RH-2703	-	-	47.4	-	-
RH-2777	-	-	1.6	-	-
RH-2778	-	-	-	-	13.9
RH-0126	-	-	3.4	-	-
RH-2631	-	-	-	15.0	-
2-propanol	-	-	44.3	-	-
Acetaldehyde	-		5.5	-	-

[1] Mean percentage of A- and B-ring labels in milk, fat, kidney and muscle, and in liver for RH-0282, 9886, 2703 and 0126. Percentage of t-butyl label in liver for other compounds.

Tebufenozide is extensively metabolized by multiple oxidative transformations in goats. In most samples the parent compound represented only a small proportion of the TRR and the same residues were found with all 3 labels, indicating that the metabolites contained the intact molecular skeleton of the parent molecule. One of the resulting alcohols, RH-9886, was found conjugated to fatty acids in milk and fat. Low levels of two other alcohol metabolites and a carboxylic acid were also found in milk. Liver was exceptional in that residues of the parent compound were not found: the major metabolites identified with the t-butyl label were 2-propanol and an approximately equal amount of RH-2703. The proposed metabolic pathways of tebufenozide in lactating goats are shown in Figure 2.

Poultry. Laying hens (6 groups of 10 hens each, 25 weeks old) were dosed by capsule with labelled tebufenozide for 7 days at a level equivalent to 30 ppm in the feed. The average feed intake was 110 g/bird/day. Another group of 10 hens served as the control. Two of the 6 groups received the A-ring label, another two the B-ring and the remaining two the t-butyl label. Samples of excreta and eggs were collected daily for the 7-day dosing period, and tissues samples were collected after all the birds were killed 24 hours after the final dose. The recovery of the ^{14}C in the A- and B-label groups was nearly quantitative, but in the t-butyl group only about 80%, probably owing to extensive degradation to produce the volatile metabolites identified in liver and possibly loss as CO_2. The total radioactivity in the tissues varied with the label, and was generally highest with the t-butyl. Significantly different TRRs from the three labels implied extensive breakdown of the molecule. The average residues found in tissues and eggs are shown in Tables 5 and 6 (Schuck and Sharma, 1996).

Table 5. ^{14}C residues in hen tissues (Schuck and Sharma, 1996).

Sample	^{14}C, mg/kg as tebufenozide, mean of 2 groups		
	A-ring label	B-ring label	t-butyl label
Liver	0.13	0.18	3.95
Fat	0.13	0.06	0.16
Thigh muscle	0.02	0.01	0.07
Breast muscle	0.006	0.0000	0.03
Kidney	0.13	0.12	0.97

Figure 2. Proposed metabolic pathways of tebufenozide in goats.

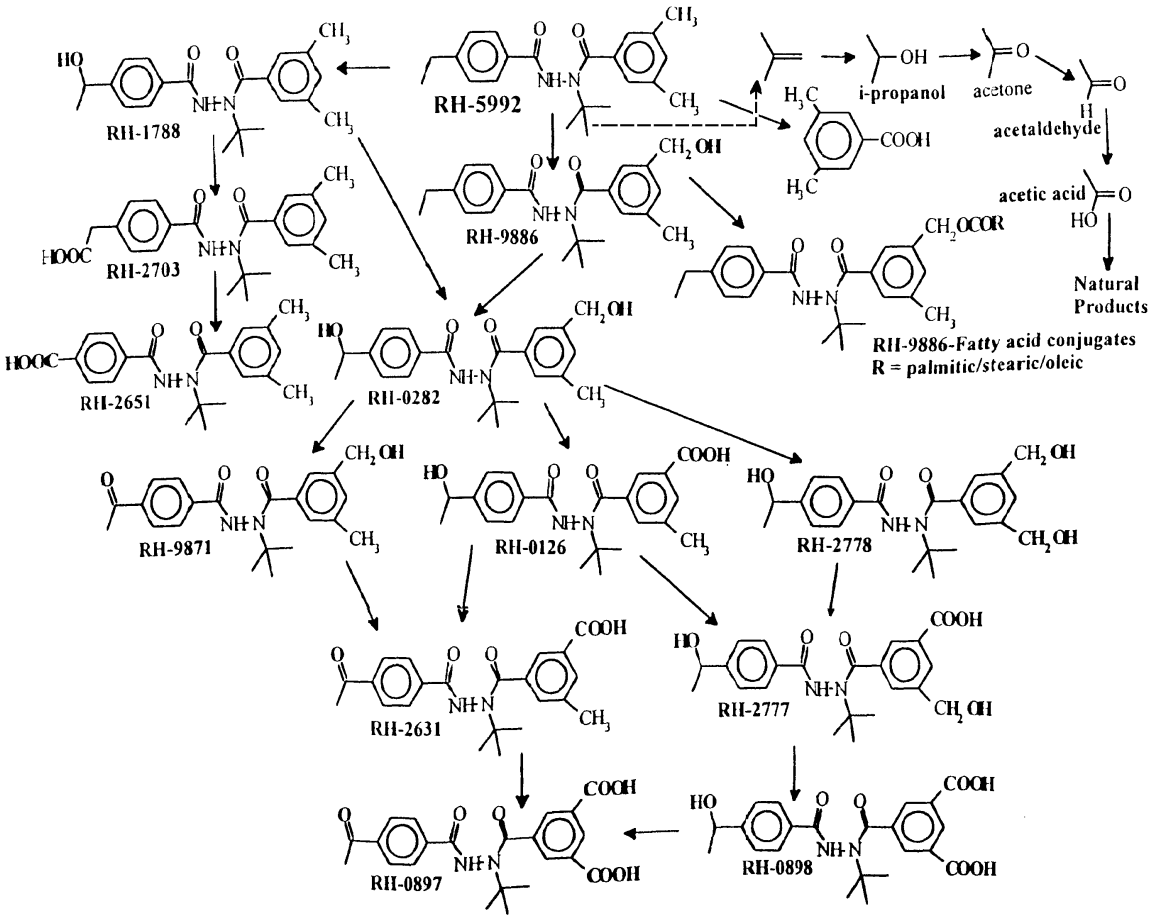


Table 6. ^{14}C residues in whole eggs.

Label	^{14}C, mg/kg as tebufenozide, mean of 2 groups						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
A-ring	0	0.02	0.03	0.04	0.06	0.06	0.07
B-ring	0	0	0.01	0.04	0.02	0.03	0.03
t-butyl	0	0.03	0.05	0.04	0.09	0.11	0.13

The hens dosed with the t-butyl label showed the highest TRR in eggs in almost all the samples collected.

The analysis of eggs showed only traces of whole-molecule metabolites such as the parent compound, RH-9886, RH-9871 and a fatty acid conjugate of RH-9886. The analysis of fat also showed a high percentage of radiocarbon from the A-ring and t-butyl labels incorporated into the fatty acids themselves.

The t-butyl labelled liver sample contained a very high percentage of the TRR as a volatile residue. More than 30% was shown to consist of two volatile components, 2-propanol and acetaldehyde, and another volatile residue was identified as acetic acid. RH-2277, RH-2778, RH-0897, RH-0126 and RH-0282 were detected in small quantities.

The residue in muscle was very low. Solvent-extractable residues in thigh muscle amounted to about 0.03 mg/kg or less and the remainder was extractable after treatment with proteolytic enzymes.

Excreta were the main source of metabolites in this study: most of the residue was soluble in organic solvents and only a small proportion was water-soluble. The compounds were identified by TLC and HPLC, and further confirmed by mass spectrometry. Tebufenozide, RH-0126, RH-0282, RH-2777 and RH-2778 were present in amounts exceeding 10%. RH-1788, RH-9886, RH-9871, RH-0897 and 3,5-dimethylbenzoic acid were also detected.

Table 7. Metabolites identified in the eggs, tissues and excreta of hens.

Compound	Residues, mg/kg				
	Eggs[1]	Liver	Fat	Muscle	Excreta
Tebufenozide	0.005		0.18	nd	Detected
RH-9886	0.001[2]				Detected
RH-0282	0.003	Detected	0.03	0.008	Detected
RH-9871	0.003				Detected
RH-9886 conj.	0.002[3]		0.01		
RH-1788/9886				0.005[3]	Detected
RH-2631				0.004[4]	
RH-2778		Detected		<LOD	Detected
Rh-0126		Detected		<LOD	Detected
RH-0897		Detected			

Compound	Residues, mg/kg				
	Eggs[1]	Liver	Fat	Muscle	Excreta
RH-2777		Detected		<LOD	Detected
3.5-dimethylbenzoic acid					Detected
acetaldehyde + 2-propanol		2.56			Detected
acetic acid		0.45			
others & unknown	0.04[5]		0.01[3]		
Natural product incorporation	>20%		>30%		

[1] A-ring label groups collected on day 5, B-ring and t-butyl label groups on day 7
[2] A-ring label only
[3] A-ring and t-butyl label
[4] A- and B-ring labels
[5] t-butyl label only

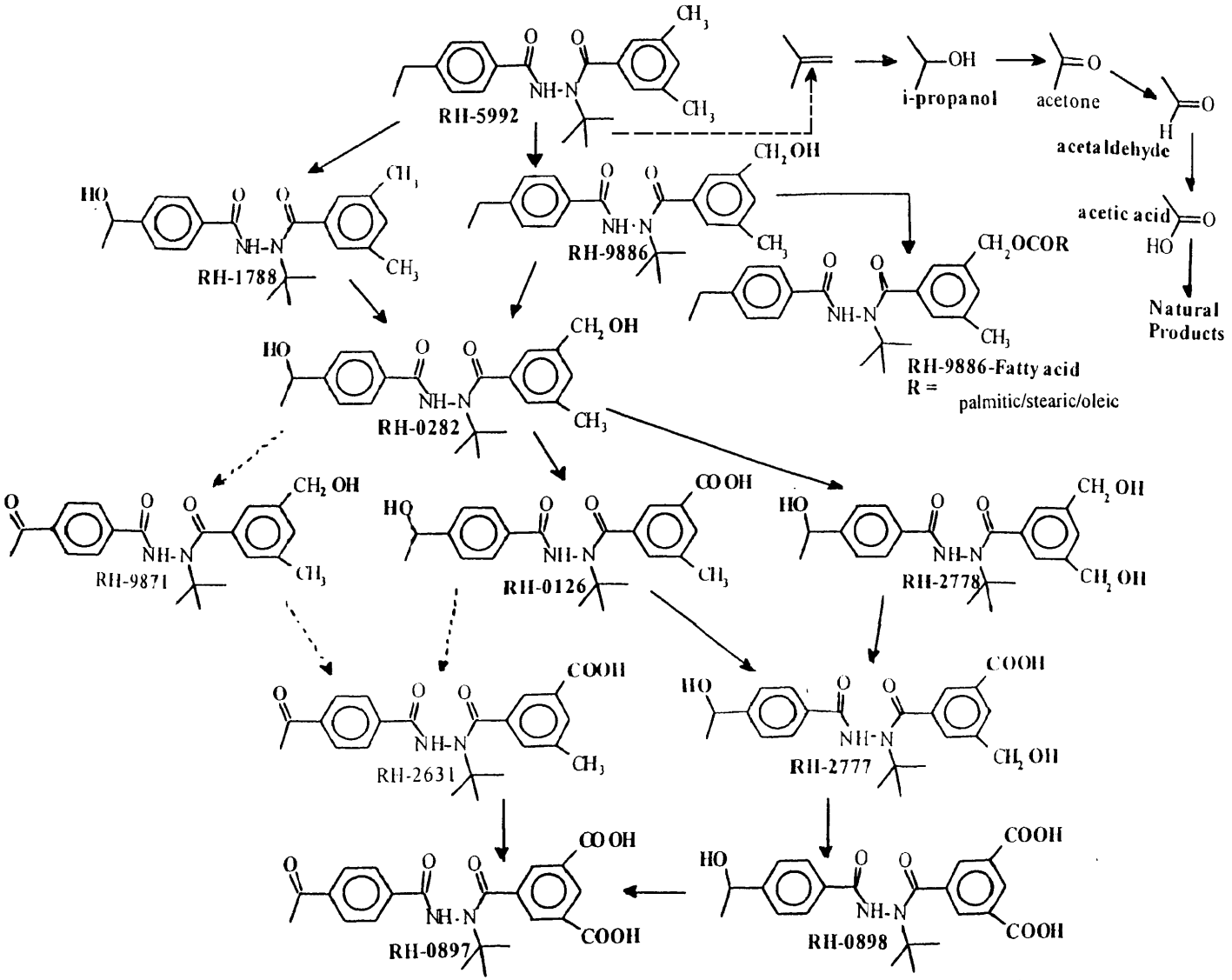
The metabolic degradation of tebufenozide in hens proceeded via oxidation of the ethyl and methyl groups of the A- and B- rings, hydrolysis of the amide portions which released the free benzoic acids and oxidative degradation of the *tert*-butyl group which resulted in the small volatile molecules 2-propanol, acetaldehyde and acetic acid. A proposed degradation pathway in hens is shown in Figure 3.

Fish. To study the kinetics of the uptake and elimination of tebufenozide, Bluegill sunfish were continuously exposed to a nominal concentration of 50 µg/l for 29 days. Each of three groups was exposed to [^{14}C] tebufenozide with one of the three labels. Thirty-five fish were then transferred from each of the three exposure aquaria to their respective depuration aquaria for a 15-day depuration period in fresh water, to determine the half-life for the loss of tebufenozide from tissues. Fish, divided into edible and inedible components, and water samples were taken at eight intervals during the exposure period and at 5-day intervals during the depuration phase, and analysed by radioassay (Christensen, 1992).

The concentrations of ^{14}C in the edible and inedible tissues and whole bodies of the fish reached a statistically determined steady state during the first day of exposure. The mean steady state concentration in the tissues and the bioconcentration factor (BCF) for each of the three radiolabels is shown below.

Label	^{14}C, mg/kg as tebufenozide, and bioconcentration factors in fish					
	Edible tissue		Inedible tissue		Whole body	
	mg/kg	BCF	mg/kg	BCF	mg/kg	BCF
A-ring	0.46	8.7	4.30	81	2.20	42
B-ring	0.32	5.9	8.3	150	3.80	70
t-butyl	0.41	8.0	4.5	88	2.20	43

Figure 3. Proposed metabolic pathways of tebufenozide in hens.



The reported half-life for depuration was less than three days for all labels. By the last (15th) day of depuration at least 90% of the radioactivity had been eliminated from the fish.

In another study to determine the rate and extent of tebufenozide bioconcentration in fish, Bluegill sunfish were also exposed for 29 days to nominal concentrations of 50 µg/l of tebufenozide labelled at the three sites as before. The concentration in the fish rapidly reached a steady state level. The BCF was 7.5 for the edible tissue and 52 for the whole body. Other fish were exposed to tebufenozide labelled in the A-ring at a nominal concentration of 300 mg/l for 14 days for the identification of metabolites.

The main residue in the fish extracts was unmetabolized tebufenozide but eight metabolites, RH-0126, RH-2777, RH-2778, RH-2652, RH-2631, RH-0282, RH-9886 and the A-ring ketone/B-ring diol of tebufenozide, were also isolated and identified (Dong and Hawkins, 1993).

The metabolic profiles in the edible and inedible tissues were the same: they are shown in Table 8. No other metabolites constituted more than 10% of the residue in any tissue. The proposed metabolic pathways for tebufenozide in fish are shown in Figure 4.

Figure 4. Proposed metabolic pathways of tebufenozide in fish.

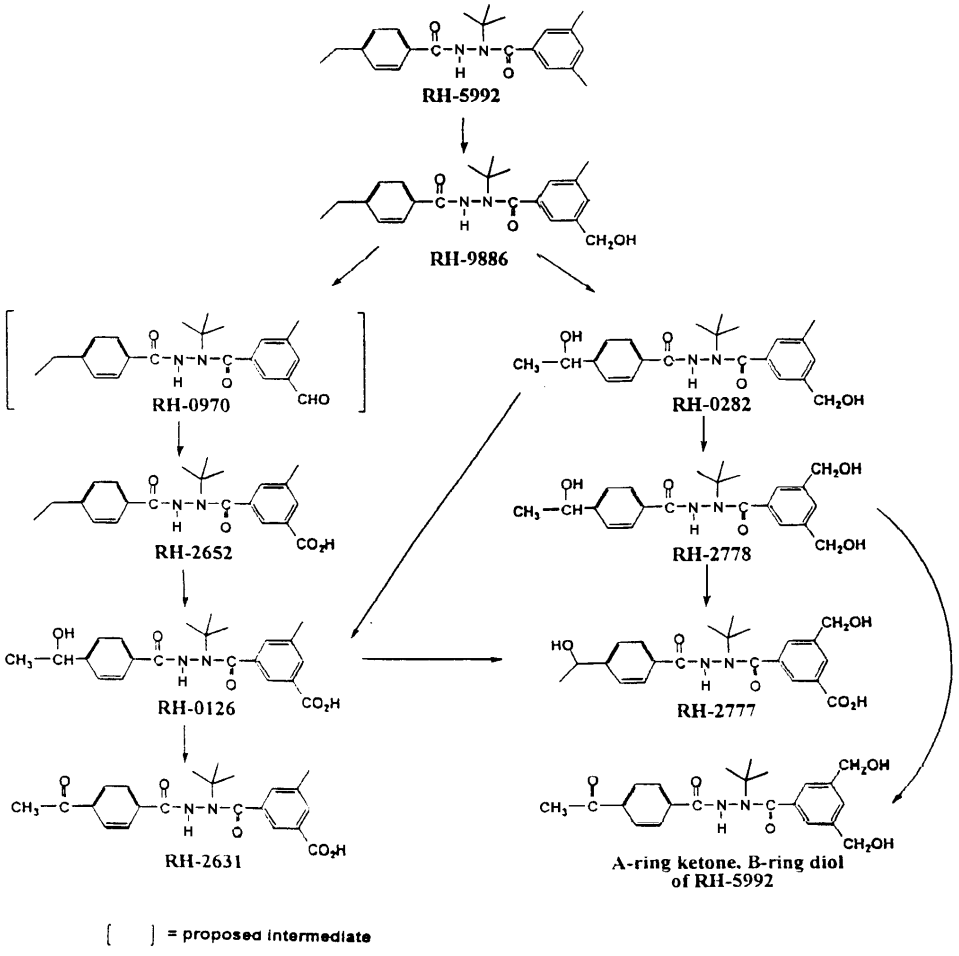


Table 8. Residues in edible and inedible tissues of fish exposed to [^{14}C]tebufenozide (Dong and Hawkins, 1993).

Compound	% of total residue			
	Edible tissue		Inedible tissue	
	t-butyl label	B-ring label	t-butyl label	B-ring label
Tebufenozide	45.6	58.8	39.4	17.3
RH-0126	10.5	17.8	19.7	21.8
RH-2652	2.3	1.9	3.6	4.2
RH-2778	5.3	3.6	2.7	5.0
RH-2777	2.9	5.0	1.8	3.8
RH-0282	2.0	0.9	0.2	0.2
RH-2631	*	*	1.3	2.5
RH-9886	*	*	*	*
A-ring ketone/ B-ring diol	*	*	*	0.2

* Below the limit of detection in fish exposed to 50 µg/l, but observed in fish exposed to 300 µg/l

Plant metabolism

Plant metabolism was studied in apples, grapes, rice, and sugar beet. All plants except apples, were treated with tebufenozide with all three radiolabels, apples only with material labelled in the A-ring.

Grapes. Grape vines (mature Concord) were treated once with [^{14}C]tebufenozide applied as a 10% EC formulation at a nominal rate of 1.2 kg ai/ha. Application was with a backpack compressed air sprayer. Samples of leaves and fruit were collected 0, 15 and 31 (harvest) days after treatment. Leaves were taken only to determine the total radioactivity, not for metabolite identification (Hawkins *et al.*, 1991).

Samples were extracted with aqueous methanol which extracted more than 98% of the ^{14}C of all three labels.

Residues in the extract were partitioned between ethyl acetate and water. The ethyl acetate fractions were analysed by HPLC and TLC. Only one radioactive component was observed and confirmed to be parent tebufenozide, which is evidently not metabolized by grapes. The three labels gave similar results, which are shown in Table 9.

Table 9. Residues in grapes and leaves after application of [^{14}C]tebufenozide to vines (Hawkins, 1991).

Sample	^{14}C, mg/kg as tebufenozide, at intervals (days)								
	A-ring label			B-ring label			t-butyl label		
	0	15	31	0	15	31	0	15	31
Leaves	187.3	41.4	93.4	136	39.3	36.4	229.4	119	115
Grapes	1.95	0.72	1.0	0.35	0.69	1.27	3.15	1.64	2.45

Rice. The metabolism of tebufenozide in rice was studied in a field experiment in California. Four 7.2 m^2 plots were lined with plastic, filled with 45 cm of a sandy loam soil and flood-irrigated after planting until final harvest. Tebufenozide, labelled in all three positions, was applied three months after planting at a nominal rate of 1.2 kg ai/ha, higher than the expected use rate. One plot served as a control. Straw, grain, paddy soil and paddy water samples were collected before and immediately after application, then at 15 days, 30 days, and at mature harvest after 64 days (Randazzo, 1992).

Straw and grain samples were chopped in a blender and ground to a fine powder in a mill. Mature grain was hulled before processing. The total radioactivity in the rice samples was determined by combustion analysis. The results are given in Table 10.

Table 10. Total radioactivity in rice treated with [^{14}C]tebufenozide (Randazzo, 1992).

Sample	Days after treatment	^{14}C, mg/kg as tebufenozide		
		A-ring label	B-ring label	t-butyl label
Straw	15	25.23	38.16	30.0
Straw	30	36.59	27.1	37.46
Straw	64	62.3	68.32	23.68
Immature grain	15	3.06	3.0	3.11
Immature grain	30	2.28	3.18	2.6
Mature grain (hulled)	64	0.33	0.40	0.29
Hulls	64	7.03	13.86	11.19

The residues in the straw, grain and hull samples were extracted with acidified aqueous acetonitrile and partitioned into organic and aqueous fractions. Most of the activity was found in the organic fraction, which was analysed after solid-phase extraction by TLC and HPLC with radiometric detection. The average recoveries were 80.6-99.1% from straw, 92-105% from grain, and 97% from hulls. The main residue in all the samples was found to be the parent compound. Four metabolites were isolated, none of which accounted for more than 10% of the sample activity. The metabolites were identified by mass spectrometry. The proposed metabolic pathway is shown in Figure 5. The metabolic profile in the final harvest samples is shown in Table 11.

Table 11. Residues in rice at harvest after treatment with [^{14}C]tebufenozide.

Sample	Compound	% of total recovered radioactivity		
		A-ring label	B-ring label	t-butyl label
Straw	Tebufenozide	78.7	77.9	71.9
	RH-0970	1.2	1.2	1.1
	RH-6595	1.1	1.3	1.3
	RH-1788	1.8	2.0	2.8
	RH-9886	1.3	1.2	1.5
Hulls	Tebufenozide	58.2	63.0	62.6
	RH-0970	1.0	1.2	1.6
	RH-6595	4.8	3.6	3.9
	RH-1788	9.4	6.6	6.9
	RH-9886	1.1	1.2	1.3
Grain	Tebufenozide	51.7	49.5	52.0
	RH-0970	1.5	1.4	1.5
	RH-6595	4.6	4.8	4.8
	RH-1788	8.8	9.4	9.8
	RH-9886	1.2	1.1	1.2

The post-extraction solids contained 9.5-17% of the total activity. The activity from one straw and one grain sample was released by mild basic hydrolysis and found to represent mainly the parent compound, with small amounts of the metabolites found in the primary extraction.

Sugar beet. Metabolism in sugar beet was studied in a field experiment in California. The three ^{14}C-labelled versions of tebufenozide were each isotopically diluted with the corresponding ^{13}C-labelled or unlabelled compound.

Each labelled tebufenozide was used as a 5% EC to treat a separate plot of sugar beet at a nominal rate of 2.24 kg ai/ha. One plot was untreated as a control. Sugar beet tops and roots were harvested at 0, 30, 61, and 120 days after treatment. The samples taken at day 0 were not analysed.

Root samples were rinsed with water, air-dried and homogenized in liquid nitrogen. Leaf samples were homogenized without rinsing. The total radioactivity in the samples, determined by combustion radioanalysis and expressed as mg/kg tebufenozide equivalent, are shown in Table 12. The residue levels in the control samples were all <0.01 mg/kg (Wu, 1993a).

Table 12. Residues in sugar beet after treatment with [^{14}C]tebufenozide (Wu, 1993a).

Sample	Days after treatment	^{14}C, mg/kg as tebufenozide		
		A-ring label	B-ring label	t-butyl label
Tops	30	2.75	4.09	2.63
	61	0.76	1.13	0.96
	120	0.44	0.27	0.56
Roots	30	0.40	0.84	0.44
	61	0.35	0.66	0.57
	120	0.16	0.23	0.13

Most of the residue, 38-59% in the final harvest beet tops and 59-83% in the roots, was found to be the parent compound, confirmed by isolation and mass spectrometry. In addition twelve metabolites were isolated and identified, none of which accounted for more than 10% of the total activity. Four of them (RH-2703, RH-2631, RH-0126, and RH-0897) were also found as conjugates in small quantities. The proposed metabolic pathways are shown in Figure 6, and the distribution of metabolites with the B-ring label in the final harvest samples is shown in Table 13.

Figure 5. Proposed metabolic pathways of tebufenozide in rice.

tebufenozide

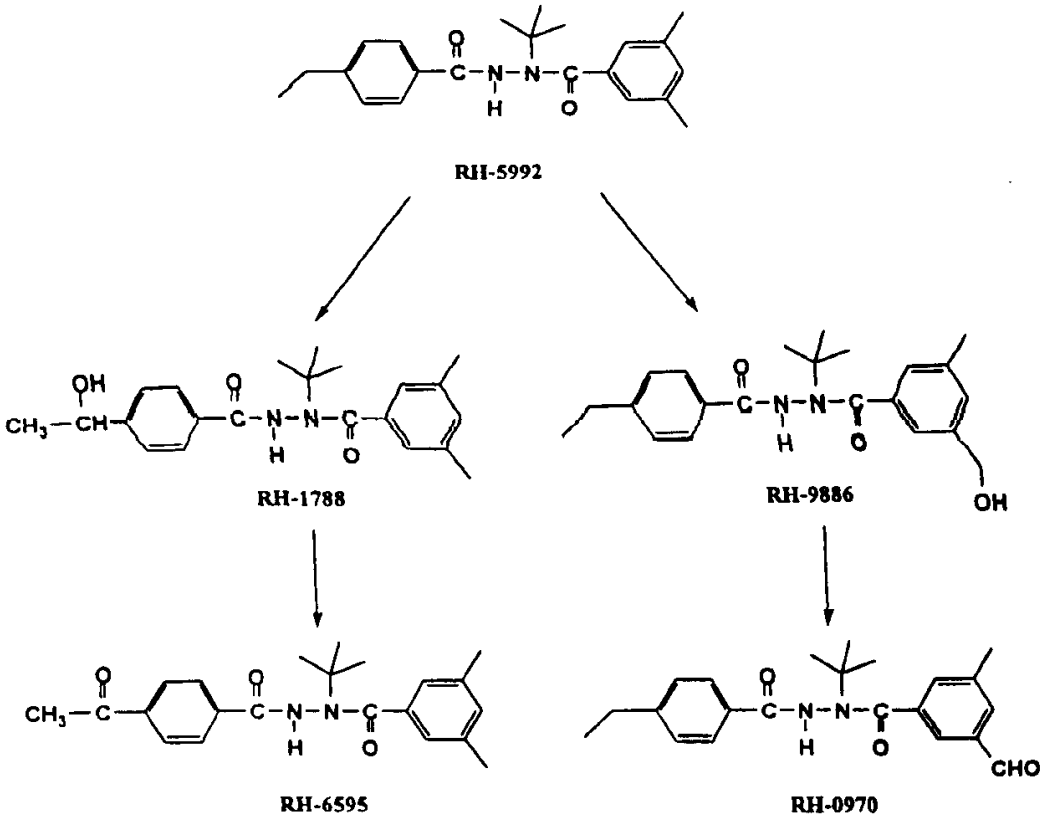
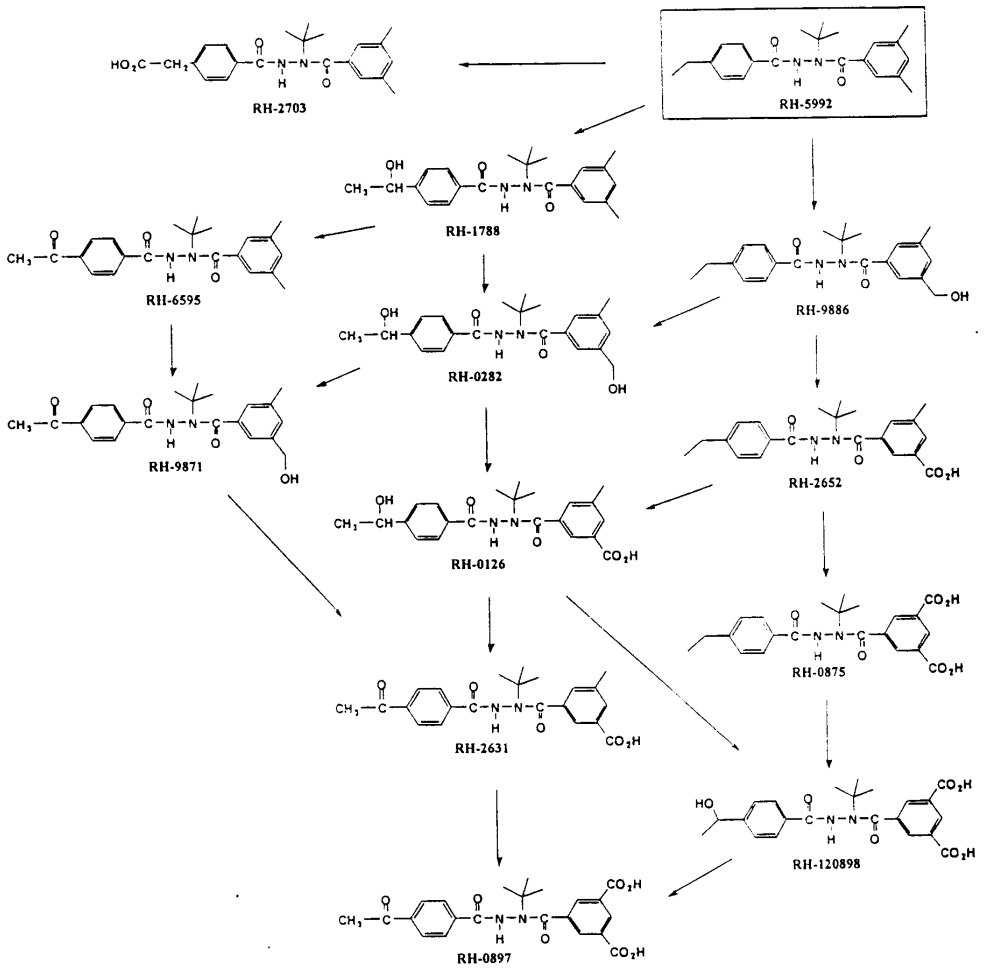


Table 13. Distribution of radioactivity in sugar beet treated with tebufenozide labelled in the B-ring (Wu, 1993a).

Compounds	% of total radioactivity	
	Tops	Roots
Tebufenozide	41.4	66.6
RH-6595	0.65	2.8
RH-9886	0.05	0.06
RH-112652	0.04	-
RH-1788	1.7	0.8
RH-2703	2.6	0.8
RH-2703 conj.	2.3	0.73
RH-9871	0.1	0.01
RH-2631	3.4	-
RH-2631 conj.	0.68	0.16
RH-0282	2.3	2.8
RH-0126	1.96	0.86
RH-0126 conj.	3.6	-
RH-0875	0.7	0.09
RH-0897	3.5	1.0
RH-0897 conj.	6.5	2.5
RH-120898	-	2.2

The main residue found in the tops and roots was the parent compound. Metabolism via oxidation of the alkyl substituents on both aromatic rings produced metabolites with varied sites and degrees of oxidation. None of the metabolites exceeded 10% of the total residue. No differences were observed between the products with the three radiolabels.

Figure 6. Proposed metabolic pathways of tebufenozide in sugar beet.



Apples. Tebufenozide can be applied to apples at various times during the whole growing season from bloom until shortly before harvest. Metabolism was studied in a field trial simulating early-season and mid-season applications at high rates, with tebufenozide uniformly labelled with ^{14}C in the A-ring. One apple tree was treated twice with a 35-day interval at 1.12 kg ai/ha giving a total of 2.24 kg ai/ha. Foliage was sampled after the first treatment, and fruit and foliage were both sampled before and immediately after, 29 days after, and 68 days after the second treatment when the fruit was ripe at the final harvest (Wu, 1993b).

Fruit and foliage samples were homogenized and the total radioactive residues determined by combustion radioanalysis. The results are given in Table 14.

Table 14. Residues in apple foliage and fruit after application of [^{14}C]tebufenozide (Wu, 1993b).

Sample	^{14}C, mg/kg as tebufenozide	
	Fruit	Foliage
Post-treatment 1	-	106
Pre-treatment 2	1.34	23
Post-treatment 2	5.34	188
29 days after 2	0.32	48
Final harvest (68 days after 2)	0.21	27

The metabolitic profiles of the fruit samples at both 29 and 68 days after the second treatment were determined by analysis of the organic extracts by TLC and HPLC with radiometric detection. Residues left in the aqueous layers (12-13% of the total) were hydrolyzed by treatment with cellulase and determined similarly. The main residue was found to be the parent compound. Four metabolites were isolated and identified, none of which represented more than about 6% of the sample activity. One metabolite, RH-1788, was found in both free and conjugated forms. RH-0282 and RH-2778 were found only as conjugates. The metabolic profiles in the fruit samples are shown in Table 15.

Table 15. Metabolite profiles in apple samples after application of [^{14}C]tebufenozide.

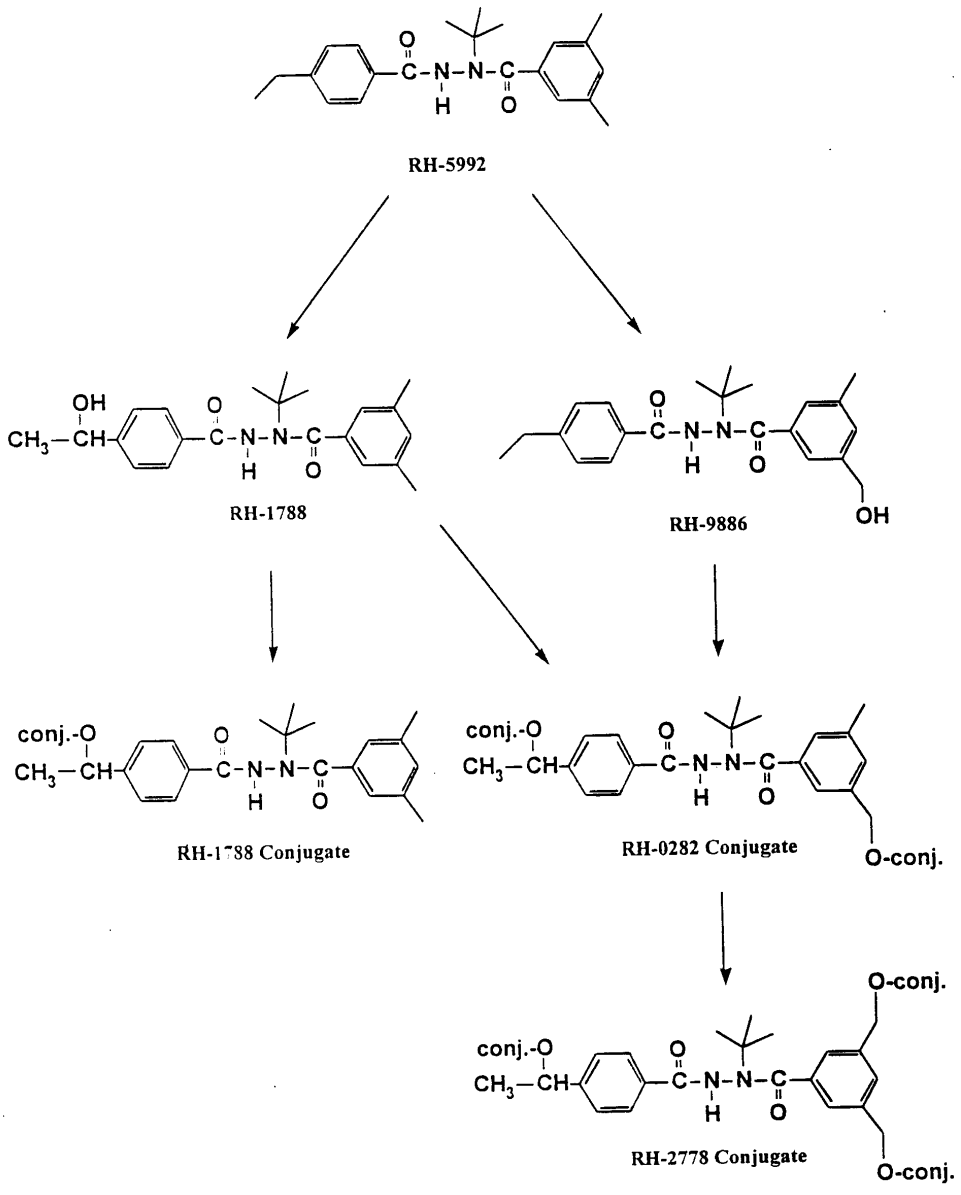
Compound	^{14}C at 29 days		^{14}C at 68 days (harvest)	
	% of total	mg/kg as tebufenozide	% of total	mg/kg as tebuconozide
tebufenozide	71.2	0.22	77.26	0.165
RH-1788	4.96	0.021	2.49	0.008
RH-1788conj.	1.52		1.47	
RH-9886	0.21	0.001	0.20	0.0000
RH-0282 conj.	6.02	0.02	4.32	0.009
RH-2778 conj	2.46	0.008	2.71	0.006
Total	86.37	0.27	88.45	0.188

The metabolic profile in the foliage sample from the final harvest was also determined. The residue was almost entirely parent tebufenozide (>93%). The only metabolite identified was RH-0282, present in both free and conjugated forms at levels of 0.24 and 0.52%, respectively, of the total activity. At least 13 unknowns were also present in the foliage, none acounting for more than 1.88% of the total activity.

The proposed metabolic pathways for tebufenozide in apples are shown in Figure 7.

tebufenozide

Figure 7. Proposed metabolic pathways of tebufenozide in apples.



Note: "conj." marks possible locations of conjugates.

Environmental fate in soil

A study of aerobic degradation was conducted with two soils, a loam and a sandy loam, at a nominal dose rate of 1 mg/kg of [^{14}C]tebufenozide. The soil samples were incubated in the dark at 25ºC for one year, in bottles with traps for CO_2 and volatile organic compounds. The three labels were used (Reynolds, 1992e).

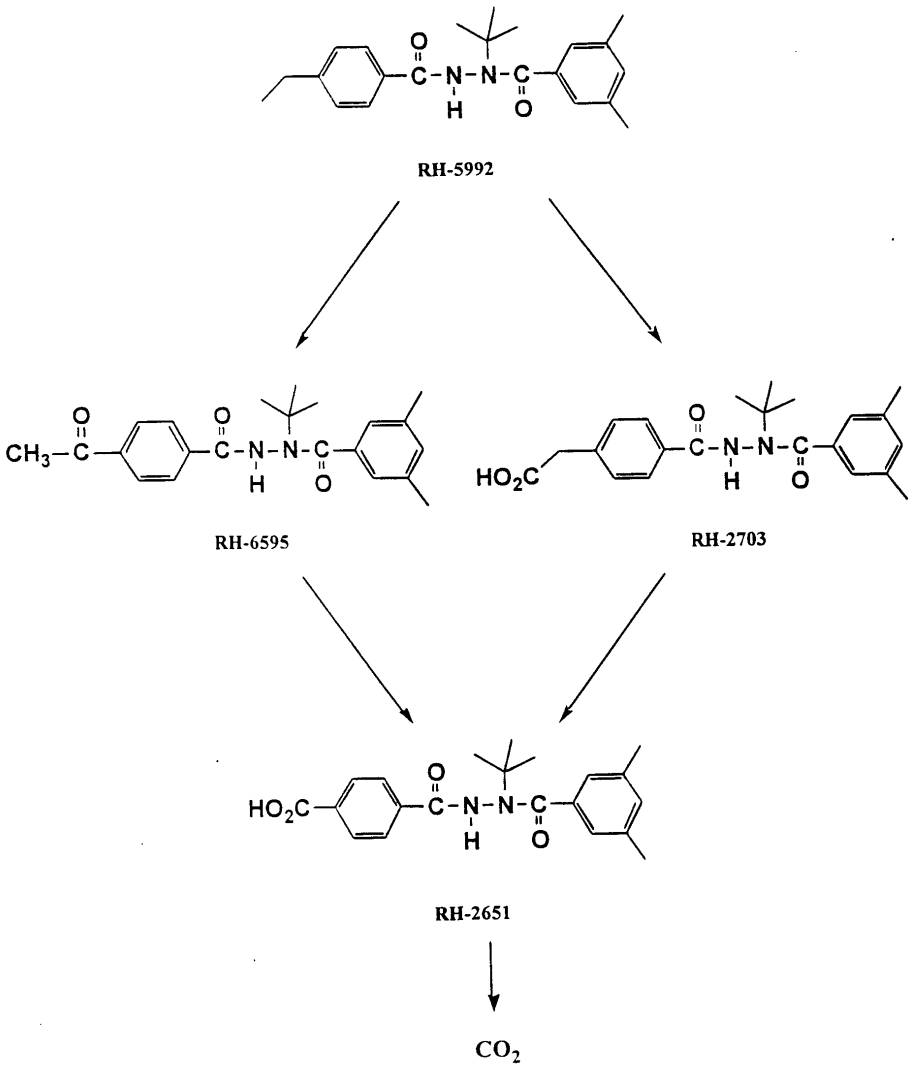
Duplicate samples were taken on days 0, 1, 3, 7, 14, 30, 60, 90, 120, 180, 270, and 365 and analysed by TLC and/or HPLC. The identities of the parent compound and major metabolites were confirmed by using a second chromatographic technique and by mass spectrometry. The average recovery of ^{14}C was >94%.

Three degradation produces were observed in addition to the parent compound and CO_2, the ketone RH-6595 and the carboxylic acids RH-2651 and RH-2703. All three are products of oxidation of the ethyl group on the A-ring of the molecule. Five other products were detected but not identified; none of them accounted for more than 5.5% of the applied activity in any sample.

The nature and amounts of the products from the three different labels were very similar in each soil, but the rates of degradation in the two soils were very different. In the loam soil (California), only 7-9% of the parent compound remained by day 365 and 54-62% of the total applied radioactivity had been converted to $^{14}CO_2$ by the end of the study. The calculated half-life of tebufenozide was 105 days. In the sandy loam soil (New Jersey) the rate of degradation was much slower; 61-71% of the parent compound remained by the end of the study and only 2-5% of the initial tebufenozide was mineralized: its calculated half-life was 704 days. It was suggested that this soil may have been atypical, but there was no explanation for the low rate of degradation.

Soil-bound residues increased gradually from all three labels as the study progressed to 12.3-16.6% in the sandy loam and 22.4-26.9% in the loam. They were characterized by various methods. Mild acidic extraction solubilized 2.6-7.0% of the total applied ^{14}C and acid hydrolysis released about 2.9-7.1%. The remainder was fractionated into humic and fulvic acids and humin, with most of the activity recovered from the humic and fulvic acid fractions. The proposed degradation pathways of tebufenozide in soil are shown in Figure 8. The half-life observed in the New Jersey soil was inconsistent with all other reported information on soil degradation.

Figure 8. Proposed degradation pathways of tebufenozide in soil.



tebufenozide

The degradation of tebufenozide was also investigated under aerobic conditions in four German soils, a low organic sand (Speyer 2.1), a high organic sand (Speyer 2.2), a sandy loam (Speyer 2.3) and a loamy sand (SLV). Soil samples dosed with [^{14}C]tebufenozide at a rate of either 0.193 kg ai/ha or 0.257 mg ai/kg of dry soil were incubated at 20 ± 2ºC in the dark for 120 days at 40% maximum water capacity. The degradation of tebufenozide was continuous in all four soils, with half-lives between 27.8 and 31.5 days and disappearance times for 90% of the initial concentration from 92.2 to 104.5 days (Schanne 1995a). The characteristics of the soils are shown below.

	Low org. sand	High org. sand	Sandy loam	Loamy sand
Organic C, %	0.98	2.50	1.11	1.07
Clay,%	3.25	4.25	9.44	10.16
Silt, %	9.46	9.16	27.71	35.23
Sand, %	87.28	86.59	62.81	54.6
Cation exchange capaciy, meq/100g	3.76	10.26	9.47	5.33
Water capacity, g/100 g dry soil	21.0	41.3	31.4	34.7
Microbial biomass, mg/100 g dry	start 12.6	start 21.1	start 19.4	start 12.6
soil	final 7.8	final 31.8	final 10.4	final 22.5
pH	5.5	5.5	1.1	1.0

The DT-50 and DT-90 values calculated for the four soils are shown below.

	DT-50, days	DT-90, days
Low organic sand	31.1	103.2
High organic sand	28.2	93.6
Sandy loam	27.8	92.2
Loamy sand	31.5	104.5

Carbon dioxide and non-extractable residues reached a maximum of 38% and 42.8% of the applied radioactivity respectively, indicating that these are the major terminal residues. At day 120, up to 12% of the applied activity was associated with the humin fraction of the soil organic matter and up to 31% with humic and fulvic acids. The degradation products RH-6595 (ketone), RH-2651 and RH-2703 (carboxylic acids) were again identified.

Photodegradation on soil. Sandy loam soil treated with 10 mg/kg of A-ring-labelled tebufenozide was maintained at 25ºC and irradiated with a xenon arc lamp for 30 days with a 12-hour light/dark cycle. The study was designed to trap any volatile materials and minimize thermal decomposition (Reynolds, 1992d).

Samples were analysed after 0, 3, 7, 21 and 30 days of irradiation. They were extracted with acidified acetonitrile/water, partitioned with methylene chloride and analysed by TLC or HPLC with radiometric detection. The half-life of tebufenozide was calculate to be 98 days under the test conditions.

A total of seven photoproducts were detected, none accounting for more than 5.3% of the applied ^{14}C in any sample. The most abundant was the ketone RH-6595; the B-ring aldehyde RH-0970 was also identified.

Adsorption/desorption was studied in five different soil types, clay, loam, loamy sand, sandy clay loam and loamy sand, with [^{14}C]tebufenozide labelled in the A-ring. The soil pH ranged from 5.6 to 7.8 and the organic matter content from 0.8% to 3.6% (Hawkins, 1992).

Replicate experiments were conducted with each of the five soils at four aqueous concentrations from 0.0516 to 0.755 mg/kg in 0.01 M calcium chloride. In each experiment, the soil and aqueous solution of the test compound were equilibrated for 24 hours, and the phases then separated and radioassayed. The soil was desorbed twice with fresh calcium chloride solution for 24 hours with radioassay after each desorption. All equilibrations were carried out at 25 ± 1ºC.

The average K_{oc} for the soils was 572, 928 and 1168 for adsorption, first desorption and second desorption respectively. On this basis the potential mobility would be classified as low.

A laboratory column leaching study was carried out in accordance with the BBA guideline. Three soils were selected: a sand with low organic matter (0.7%), a sand with high organic matter (2.29%), and a sandy loam with an organic matter content of 1.34% (Knoch, 1993). After sieving, the soils were packed in glass columns of 5 cm diameter and 40 cm length to produce a soil depth of 30 cm. Formulated tebufenozide was added at 37.91 µg of ai to each soil column to simulate the application of 0.192 kg ai/ha. Leaching was by application of an artificial rainfall of 393 ml within 48 hours (simulating a rainfall of 200 mm). The leachate was collected from the first and second 24-hour periods and analysed.

The tebufenozide in the leachate from the sandy soil after 48 h was <2% of that applied. In sandy loam soil the amount of tebufenozide in the first 24 h leachate was <2% of that applied, and over the entire period 0.95 Ì g was recovered in the leachate, 2.5% of the added tebufenozide. In the loamy sand column no significant amounts of tebufenozide were observed in the leachate during the first 24h. By the end of the leaching period the leachate from one of the duplicate columns contained 5.5% of the applied tebufenozide and that from the second column contained none.

A column leaching study was carried out with aged residues in clay loam, sand, sandy loam and loam. Radiolabelled tebufenozide was mixed with the soils at a nominal rate of 1 mg/kg and the mixtures aged for 30 days, yielding the residues shown below (Reynolds, 1992b).

Compound	% of applied ^{14}C			
	Clay loam	Sand	Sandy loam	Loam
Tebufenozide	52.3	71.4	52.7	66.4
RH-6595	4.11	3.05	3.65	2.38
RH-2651	5.98	2.06	15.22	0.18
RH-2703	1.21	4.87	2.62	0.95

Duplicate glass columns were filled to a height of 30 cm with each of the sieved soils. The corresponding soil with the aged residue was applied to the top of each column and the columns were eluted with 0.01 M calcium chloride solution. The columns were divided into 6-cm sections, and the eluates, soil sections and dose plugs were radioassayed. The distribution of the radioactivity is shown in Table 16.

Table 16. Distribution of radioactivity in aged soil columns (Reynolds, 1992).

Sample	% of ^{14}C recovered from column			
	Clay loam	Sand	Sandy loam	Loam
Treated plug	52.0	46.9	60.2	91.8
Segment 1 (0-6 cm)	25.2	3.36	3.85	5.53
Segment 2	11.6	4.81	3.50	1.58
Segment 3	2.44	5.66	3.84	0.22
Segment 4	0.71	6.55	3.5	0.10
Segment 5	0.27	6.96	2.27	0.02
Eluate	7.76	25.8	22.9	0.77

Analysis of the eluates indicated that the two carboxylic acids were mobile in all four soils, and the parent tebufenozide was somewhat mobile in sand.

A similar study was carried out with a sandy soil with a low organic matter content. The soil was treated with [^{14}C]tebufenozide and aged in air for 40 days before leaching. After ageing, 17.5% of the radioactivity was unextractable, 11.3% had been converted to carbon dioxide and 0.3% had been volatilized. The remainder of the extractable applied ^{14}C was associated with tebufenozide (46.7%), RH-6595 (6.6%), RH-2651 (9.1%), RH-2703 (4.6%), and two unknowns present at 1.6% and 5.2 %.

After one day of leaching the highest level of radioactivity in the eluate was 0.3%, and after the second day the average activity was 6.9% of that applied. Analysis of the eluates showed that no tebufenozide or ketone (RH 6595) was present in the leachate, whose activity was due to the presence of two carboxylic acids (Schanne, 1995b).

Field dissipation studies were carried out in the USA, Canada, Japan and Germany to determine the persistence and mobility of tebufenozide and its soil degradation products.

In two trials in California, the test material was applied directly to bare sandy soils with low organic matter at a rate of 1.12 kg ai /ha. Under these conditions, residues of tebufenozide and its products were found only in the top 30 cm of the soil: no downward movement of the compounds was observed. The degradation of tebufenozide at the two sites was similar. The half-lives determined from the exponential decline were 53 and 39 days. The trials demonstrated that even when tebufenozide was applied at the maximum rate and to unprotected soil it did not persist and residue levels wwere below 0.01 mg/kg after one year or less (Hawkins, 1993).

Two similar studies were conducted at two other US locations. The soil at a New York site was a sandy loam at the top, becoming a loam below 15 cm and a silt loam below 60 cm; the soil at a Washington site was a low-organic sand. Each site received four treatments 14 days apart of 0.56 kg ai/ha giving a total of 2.24 kg ai/ha. All treatments were made to bare soil using the 2SC formulation of tebufenozide. Residues of tebufenozide and its degradation products were found only in the top 45 cm. The half-lives determined from the exponential decline were 52 and 31 days at New York and Washington respectively. This study also shows that tebufenozide residues are near or below 0.01 mg/kg after a year or less (Hawkins, 1994).

Field dissipation studies were conducted at two sites in Japan, with cultivated loam soils of high (6.49%) and low (1.45%) contents. At each site tebufenozide was applied three times at one-week intervals at a rate of 0.4 kg ai/ha. Soil from the top 10 cm was sampled on days 0, 7, 14, 30, 55 or 60, and 90 and analysed for tebufenozide and its degredation products. The half-life of tebufenozide was 6 days in the soil with high organic matter and 19 days in that with low organic matter (Yajima, 1992).

Other field dissipation studies were carried out at four different Japanese sites under paddy conditions, with tebufenozide applied at a rate of 0.3 kg ai/ha. Soil from the top 10 cm was sampled according to a regular schedule for a total of 240-365 days. The half-lives in the four soils ranged from 4.2 to 30 days (Chong, 1992).

The dissipation of tebufenozide under field conditions was also investigated at four locations in Germany. The test substance was applied at a rate of 0.192 kg ai/ha to bare soil and samples were collected from 0-10 cm, 10-20 and 20-40 cm depths before and after application, and approximately 1 week, 2 weeks, and 1, 2, 3, and 5 months after application. The soils were a loamy sand, two sandy loams and a sandy silt loam. Soil samples were analysed for residues of tebufenozide and its products, with an LOD for each of the analytes of 0.01 mg/kg. The DT50 and DT90 values for tebufenozide in each soil are shown below (Sochor and Holzwarth, 1995).

	Disappearance times of tebufenozide, days			
	Loamy sand	Sandy loam	Sandy loam	Sandy silt loam
DT 50	108	10	13	5
DT 90	968	112	43	137

Three dissipation studies were conducted in different regions of Canada in orchard soils. The soils at the three sites were characterized as loamy fine sand, silty loam, and very fine sand. In all the trials the test compound was applied four times at a rate of 0.28 kg ai/ha. Samples taken at intervals 0 to 368 days were divided into 0-7.5 cm, 7.5-25 cm, 25-50 cm and 50-60 cm sections for analysis (MacLeod, 1995a,b.c).

The first study, with loamy fine sand, was in a mature orchard of malus trees with a ground cover of bunch-type grass. The concentration of tebufenozide shortly after application was low, the maximum concentration being reached in the top 7.5 cm on day 122, probably owing to test material moving from the vegetation into the soil (MacLeod, 1995a).

The trial with silty loam was adjacent to an apple orchard. The concentration of tebufenozide in the top 7.5 cm after the last application was 0.29 mg/kg and was below the limit of determinatiaon (0.02 mg/kg) in the sample taken 284 days after the last application. The half-life of tebufenozide was 75 days.

The third trail was in an area typical of the fruit growing region. Applications were directly on to the bare soil surface. Residues of tebufenozide on day 0 averaged 1.29 mg/kg in the top 7.5 cm and decreased to 0.12 mg/kg in the sample taken 368 days after the last treatment. The calculated half-life was 135 days.

Environmental fate in water/sediment systems

The solubility of tebufenozide in water was determined by the shake-flask method, using aqueous mixtures of the labelled test compound to provide concentrations of 5, 10, 25 and 50 mg/l if all the tebufenozide dissolved. The concentration after the final equilibrium averaged 0.83 mg/l in the four tubes (Kelly, 1992b).

The hydrolysis of tebufenozide was studied in sterile aqueous solutions buffered to pH 5, 7, and 9, containing 0.5 mg/l of [^{14}C]tebufenozide and less than 1% by volume of acetonitrile, and maintained at 25 ± 1ºC for 30 days in the dark. Aliquots of the solutions were analysed after 0, 3, 7, 14, 21, and 30 days for the test compound and possible hydrolysis products by solid-phase extraction, LSC, TLC and HPLC.

The total recoveries of applied radioactivity averaged 105-109% for the three systems. The only significant radioactive compound in the solutions was confirmed by normal-phase TLC and reverse-phase HPLC to be tebufenozide. No hydrolysis products were detected (Reynolds, 1992a).

The photolysis of tebufenozide, labelled in the A-ring, was studied in a 0.5 mg/l solution in natural pond water maintained at 25 ± 1ºC. The sample tubes were irradiated with a Xenon lamp, with 12-hour light/dark cycles, for 30 days. CO_2 and other volatile materials in the head-spaces of the sample tubes were retained in a series of traps. Duplicate sample tubes were removed from the photolysis chamber and the solutions analysed for the test compound and possible photoproducts at 0, 3, 7, 14, 21 and 30 days.

Analyses were by partition with organic solvent and liquid scintillation counting of the two phases, followed by TLC and HPLC of the organic phase with radiometric detection. No aqueous phase contained more than 5% of the applied radioactivity and no significant amount of volatile material was formed.

Tebufenozide was degraded with a half-life of 67 days under the conditions of the experiment. The major product was the ketone RH-6595, produced at a maximum level of 5.3% of the original ^{14}C. Eight other photoproducts were detected, none exceeding 3.5% of the applied activity in any sample. Almost no degradation occurred in the dark control. Recoveries of the applied radioactivity were 96-106% (Reynolds, 1992c).

In a study of aerobic aquatic degradation two hydorsoils and their respective paddy waters were incubated with tebufenozide labelled at all three sites at a nominal rate of 1 mg/kg in the dark at 25 ± 1ºC for one year. The sediments were Arkansas silty clay and California clay loam (Reynolds, 1992g). The characteristics of the two systems are shown in Table 17.

Table 17. Characteristics of water/sediment systems.

	Arkansas silty clay		California clay loam	
	soil	water	soil	water
Sand, %	12		30	
Silt, %	42		34	
Clay, %	46		36	
Organic matter, %	2.0		2.6	
pH	6.9	6.7	7.8	6.9
O_2 (dissolved, ppm)		8.10		6.85

Duplicate samples were taken for analysis on days 0, 1, 3, 7, 14, 30, 60, 120, 180, 270 and 365. Volatile compounds were trapped throughout the study. At each sampling, the soil and supernatant water were separated by decanting and centrifuging and analysed separately. The radioactive components in each phase were extracted and determined by TLC and/or HPLC, and the identities of the parent compound and the major products were confirmed by mass spectrometry. The distribution of radioactivity and the nature and amounts of the products from the three labels were similar. The results are summarized in Tables 18 and 19.

Table 18. Average distribution of radioactivity at various intervals in Arkansas and California soils.

Days incubation	% of ^{14}C							
	Arkansas				California			
	Supernatant	Solids	PES[1]	Traps	Supernatant	Solids	PES[1]	Traps

Days incubation	% of ^{14}C							
	Arkansas				California			
	Supernatant	Solids	PES[1]	Traps	Supernatant	Solids	PES[1]	Traps
0	55.8	43.4	0.74	0	53.4	45.9	0.69	0
1	45.2	48.8	0.5	0.02	39.7	63.0	2.4	0.01
3	36.4	54.6	3.18	0.04	40.6	60.4	3.7	0.01
7	26.0	64.6	2.1	0.05	22.4	75.1	5.9	0.02
14	17.5	75.1	5.8	0.05	19.8	78.4	7.0	0.03
30	16	74.7	7.5	0.27	12.7	74.8	15.3	0.06
59-60	21.3	57.4	15.2	1.2	13.0	79.7	12.9	0.4
88-90	23.9	64.5	8.2	2.9	15.9	78.3	9.3	1.9
120	24.4	59.4	10	5.2	19.1	65.0	14.9	6.9
179	21.4	51.2	13.7	10.5	18.4	48.4	20.3	18.6
270	31.1	29.9	15.4	20	11.7	23	24	34.2
360	29.2	24.5	20.7	30.1	5.56	11.5	22.7	47.4

[1]Post-extraction solids

Three major compounds were observed in the study in addition to tebufenozide and CO_2. These were the ketone RH-6595 and the two carboxylic acids RH-2651 and 2703, all products of oxidation of the ethyl group on the A-ring of molecule. Five additional degradation products were also found and characterized by their chromatographic behaviour but not identified. None of these accounted for more than 5.7% of the applied activity in any sample. Up to 47% of the total applied ^{14}C was converted to $^{14}CO_2$ by the end of the study.

Sediment-bound residues increased gradually as the study progressed to 19-23% of the ^{14}C from all three labels in both hydrosoils. The bound residues after 358-366 days were characterized by means of various techniques. Mild acidic extraction solubilized 5-6% of the total applied ^{14}C and acid hydrolysis released about 4%. The remainder was fractionated into humic and fulvic acids and humin, with the activity predominantly in the fulvic acid fraction.

The half-lives of the parent compound under the aerobic aquatic test conditions (average of the three labels) were 101 days in the California clay loam and 99 days in the Arkansas silty clay.

Table 19. Distribution of residues in the aquatic degradation of tebufenozide.

Day	Percentage of ^{14}C present as				
	Tebufenozide	RH-6595	RH-2651	RH-2703	CO_2
Arkansas silty clay, A-ring label					
0	98.29	0	0	0	0
1	91.70	0.13	0	0	0.02
3	89.53	0.92	0	0	0.04

Day	Percentage of ^{14}C present as				
	Tebufenozide	RH-6595	RH-2651	RH-2703	CO_2
7	88.11	2.26	0	0	0.05
14	90.01	0	0	0.07	0.05
30	76.77	5.52	0	0	0.27
59	60.17	2.14	2.63	8.71	1.23
90	64.31	4.88	4.16	8.78	2.94
120	49.05	6.92	9.49	9.43	5.19
178	30.78	6.56	14.26	11.14	10.53
269	12.71	2.56	32.07	2.59	20.02
366	7.02	1.07	35.45	0	30.08
California clay loam, B-ring label					
0	98.48	0	0	0	0
1	96.92	0.29	0	0	0
3	94.92	0.70	0	0	0.01
7	95.67	0.32	0	0	0.01
14	91.46	0.42	0	0	0.02
30	86.63	0	0	0	0.05
60	80.80	5.10	0	0	0.35
88	64.99	8.10	2.57	4.74	2.04
120	58.18	4.99	2.86	4.62	6.09
179	31.11	4.26	8.46	4.66	18.08
270	15.17	2.50	6.40	1.11	35.78
358	7.94	0.78	1.88	0.05	47.00

A companion study of anaerobic aquatic degradation with a flooded silt loam hydrosoil, was again in the dark at 25 ± 1ºC for one year at a nominal rate of 1 mg/kg of tebufenozide, uniformly labelled with ^{14}C in the A-ring and also with ^{13}C in the two methyl groups on the B-ring to aid mass spectral identification if needed. Duplicate samples were taken for analysis on days 0, 3, 7, 14, 30, 60, 90, 120, 179, 270 and 365. Volatile products were trapped throughout the study (Reynolds, 1992f).

Two significant compounds were found apart from tebufenozide and CO_2: the ketone, which reached a maximum of 8.0% of the applied activity at day 179, and the carboxylic acid RH-2651 which reached 11.6% at day 120. Eight other compounds were found and characterized. One was the carboxylic acid RH-2703, the others were not identified. None of these minor products exceeded 4% of the applied activity in any sample. The calculated half-life of tebufenozide was 179 days.

METHODS OF RESIDUE ANALYSIS

Analytical Methods

A number of methods have been developed to analyse specifically for tebufenozide in various crops and processed fractions. They depend on GLC or HPLC and have been validated.

Fruits. An HPLC method was used to determine tebufenozide residues in apples, grapes, kiwifruit, apple juice and wine. Apples are extracted by blending with 0.1 N HCl/methanol (1:9). The extract is partially purified by partitioning first with hexane, which is discarded, then with methylene chloride. The methylene chloride layer is concentrated and further purified by column chromatography on basic alumina or Florisil. The residue is determined by HPLC with UV detection. The limit of determination (LOD) in apples was 0.02 mg/kg, with an average recovery of 81.3 ± 11.5% (Deakyne *et al.*, 1994b). The LODs were 0.01 mg/kg in grapes and 0.005 mg/kg in wine with recoveries of 87 ± 11.3% and 81 ± 12.4% respectively (Deakyne *et al.*, 1994a). The average recovery from kiwifuit was 108 ± 5.7%.

In a revised version of this method (Deakyne *et al*., 1995) the identity of the residue was confirmed by HPLC-MS after the same extraction and purification procedure. This revised method was validated for kiwifruit with a limit of detection of 0.01 mg/kg and was used to analyse kiwifruit from the 1994-5 residues trials in New Zealand (Deakyne *et al.*, 1995).

Schuld and Holzwarth (1994a) described a method for the determination of tebufenozide in apples, grapes, apple juice and wine by GLC. Samples (25 g) of apples and grapes are extracted with acetone and the acetone removed in a rotary evaporator. The remaining aqueous phase is extracted with n-hexane and the extract cleaned up by silica gel chromatography. Juice and wine (20 g) are concentrated on an Extrelut® column which is eluted with n-hexane. Tebufenozide is methylated with methyl iodide and the derivative extracted from the reaction solution with n-hexane. The *N*-methyl derivative is determined by gas chromatography with a nitrogen-phosphorus detector (NPD). The lower level of the practical working range for the method is 0.02 mg/kg for apples and grapes and 0.01 mg/kg for juice and wine. Recoveries ranged from 72% to 128% for grapes, 74% to 129% for apples, 77% to 109% for wine, and 63 to 159% for apple juice with mean recoveries of ≥80% for all samples.

The method was modified to complete the determination by GS-MS rather than GLC with an NPD. The limit of determination was 0.02 mg/kg.

In another GLC method (Mellet, 1993a) samples of grapes, must and wine are again extracted with acetone and, after evaporation of the solvent, extracted from the remaining aqueous phase with hexane. This extract is partitioned with acetonitrile and the residues are further purified on a silica gel column. Tebufenozide is then methylated with methyl iodide in the presence of sodium hydride and dimethyl sulfoxide. The *N*-methyl derivative is again determined by gas chromatography with an NPD. The limit of determination was 0.01 mg/kg in all three types of sample, with recoveries from grapes, must, and wine of 71-128%, 66-130%, and 54.5-116% respectively.

In a method for kiwifruit (skin and edible pulp) described by Tillman (1995d) residues are extracted with acetone and cleaned up as in Mellet (1993a), but tebufenozide is determined (without derivsatizaion) by HPLC with an isocratic mobile phase and UV detection at 220 nm. The method was validated for kiwifruit with an average recovery of 96.9% at 0.2 mg/kg.

Nuts. In a method described by Cui and Desai (1994a) residues of tebufenozide are Soxhlet-extracted from pecans with methanol. Sodium chloride solution is added to the extract and a hexane partition removes the oils. The residues are then extracted into methylene chloride. The solvent is evaporated and the residue cleaned up on a basic alumina column. Solid-phase extraction on carbon provides an optional additional clean-up. Tebufenozide is determined by HPLC with UV detection at 240 nm. Mass spectrometry can be used for confirmation. The average recovery was 81.9 ± 10.2%, with a reported limit of determination of 0.01 mg/kg.

A method for the analysis of walnuts described by Cui *et al.* (1993a) is similar to that for pecans, with further clean-up. The organic phase is concentrated to dryness and cleaned up on three columns, the first of carbon, the second a C-18 phase, and the third basic alumina. The final eluate is dried and dissolved in 30% acetonitrile/water for gradient HPLC (mobile phase A: 10% methanol in water; mobile phase B: acetonitrile) on an Adsorbosphere C-18 5µ column with a UV detector. The limit of determination was 0.01 mg/kg and recoveries from fortified samples averaged 91.1 ± 11.8%. A Supelco LC-DP 5µ column is used for confirmation.

This method was revised, first to incorporate the LC-DP confirmatory method with a new solvent system (Cui *et al.,* 1994) and subsequently to include an additional confirmatory method using HPLC-MS (Cui *et al.,* 1995).

Vegetable crops (cabbage, lettuce, spinach, broccoli, celery and mustard greens). Residues can be determined according to Chen *et al.* (1993). Samples are blended with 0.1N HCl/methanol (1:9). The extract is partitioned with methylene chloride and the concentrated methylene chloride layer is cleaned up chromatographically. Extracts of lettuce, cabbage, mustard greens and spinach are cleaned up on a single basic alumina column. Broccoli and celery extracts are passed through three small solid-phase extraction (SPE) tubes which contain successively carbon, basic alumina, and cyano adsorbent. Tebufenozide is determined by HPLC with UV detection. The limit of determination (LOD) was 0.01 mg/kg for all vegetables except celery which had a LOD of 0.05 mg/kg.

This method also was revised to add the options of a basic alumina chromatography clean-up step and HPLC-MS confirmation method (Chen *et al.,* 1994a).

Ishii and Higuchi (1993) described an HPLC method to analyse Chinese kale. Samples are extracted with acetone, which is evaporated. The residue is taken up in dichloromethane and cleaned up by Florisil and alumina column chromatography. The reported detection limit was 0.01 mg/kg, with an average recovery of 95.2%.

Chilli peppers were analysed by the method described above with an additional Florisil column before the alumina column chromatography (Ishii and Higuchi, 1994). The reported detection limit was 0.01 mg/kg and the average recovery was 96.6%.

Rice (grain and straw). A method for residues of tebufenozide and two metabolites in rice grain was described by Komatsu and Yabusaki (1992a). Samples (10g) are first soaked in water for two hours before extraction with acetone. A portion of the acetone extract is concentrated to 10 ml before being dissolved in water and cleaned up on a column of granular diatomite adsorbent. The fraction containing tebufenozide, the ketone RH-6595 and the corresponding alcohol RH-1788 is dried under nitrogen, partitioned with hexane and acetonitrile, and cleaned up further by silica gel chromatography. RH-6595 is reduced to RH-1788 with sodium borohydride. After the reduction step the combined residues are methylated with methyl iodide and sodium hydride in a mixture of benzene and dimethyl sulfoxide. The *N*-methyl-tebufenozide and the *N*-methyl-*O*-methyl-RH-1788 are determined by GLC with an NPD. The limit of detection of all three compounds was 0.005 mg/kg. The average recoveries of tebufenozide, RH-1788 and RH-6595 were 72%, 96% and 96% respectively. Residues of RH-1788 and RH-6595 would of course in practice be reported as one combined value (quantified as RH-1788).

The method for rice straw (Komatsu and Yabusaki, 1992b) is virtually identical to that used for the grain except for the sample size: 5 g of straw is extracted. The limit of detection of all three compounds in straw was 0.04 mg/kg, with average recoveries of tebufenozide, RH-1788 and RH-6595 of 78%, 82%, and 74% respectively.

Tea and brewed tea. An HPLC method for analysing dry and brewed tea was described by Ishii (1995). Dry samples are extracted with water/acetone, filtered, partitioned with dichloromethane, and the extract transferred to acetone. The acetone solution is cleaned up by coagulation and filtration through Celite, then extrated with dichloromethane before clean-up on Florisil and alumina columns. The limit of detection was 0.05 mg/kg and the average recovery 86.7%. Brewed tea is cleaned up by precipitation with zinc acetate and extraction with dichloromethane, followed by Florisil and alumina column chromatography. The residue is again determined by HPLC. The limit of detection was 0.01 mg/kg and the average recovery 91.6%.

A GLC method to analyse tea and brewed tea for tebufenozide, RH-6595 and RH-1788 described by Komatsu and Yabusaki (1993) is similar to the same authors' method for rice. Dry tea is extracted with acetone and cleaned up by chromatography on a porous kieselguhr column, coagulation, and chromatography on silica gel. RH-6595 is reduced to RH-1788 with sodium borohydride, tebufenozide and RH-1788 are methylated with methyl iodide, and the methylated compounds are partitioned with hexane. After clean-up by silica gel column chromatography, the residues are determined by GLC with an NPD. The limit of detection was 0.01 mg/kg for both tebufenozide and RH-1788 and the average recoveries of tebufenozide, RH-1788, and RH-6595 were 95%, 86%, and 100% respectively. Filtered brewed tea is partitioned with dichloromethane and cleaned up on a silica gel column. The analysis is completed by reduction, methylation, clean-up and GLC as before. The limit of detection was again 0.01 mg/kg for both analytes, with average recoveries of 90%, 74%, and 72% for tebufenozide, RH-1788, and RH-6595 respectively.

Soil. Tebufenozide and its degradation products, the ketone RH-6595 and the carboxylic acids RH-2703 and 2651, are easily extractable from soil with methanol/0.5N HCl (3:1). This extract is partitioned twice with dichloromethane. After concentration, the acids are esterified with diazomethane and the mixture is cleaned up on a Florisil column. Tebufenozide and its metabolites are eluted with ethyl acetate/hexane/acetone. The eluant is evaporated and the residue reconstitued in methanol/water for analysis by HPLC. Recoveries at fortification levels from 0.02 to 0.1 mg/kg were 85-87% for tebufenozide and above 90% for its metabolites (MacLeod, 1995a).

Water. Tebufenozide residues are extracted from water with methylene chloride after the addition of sodium chloride. The methylene chloride is evaporated and the residue dissolved in the mobile phase for determination by HPLC. An extra clean-up by silica gel chromatography may be necessary for stream waters. The mean recovery from all types of water was 98.4 ± 8.8% at fortification levels of 0.1 to 5 ì g/l. The reported LOD was 0.1 ì g/l (Deakyne *et al*., 1992).

Stability of residues in stored analytical samples

The stability of tebufenozide in stored analytical solutions was investigated in the USA (Chen *et al.*, 1994e). Standard solutions of tebufenozide at concentrations from 0.01 to 1.0 mg/l in either methanol/water or acetonitrile/water were stored for various periods and compared with freshly made standards by HPLC injection and integration of the peaks. Statistical analysis of the results indicated that working standards of tebufenozide were stable for a minimum of 6 months.

The stability of tebufenozide residues was studied in samples of apples, apple juice, grapes, wine, rice, walnuts and lettuce.

Apples and apple juice. A study of the stability of residues of tebufenozide in apple samples during frozen storage is in progress. Untreated apple samples were homgenized, and control and fortified samples were stored at -15 ± 1ºC. The results reported so far (Deakyne and Chen, 1995a) show that tebufenozide is stable for at least six months in apples when stored frozen. The percentage of the residue remaining aftere six months storage ranged from 90.8% to 108% with no indication of a systematic decrease with time.

Additional information on the stability of tebufenozide residues in apples during frozen storage (-15ºC) was obtained by analysing field-treated samples, storing them for two additional years and re-analysing the same samples (Deakyne, 1995b; Burnett *et al*., 1994). The results are shown below.

Sample	First analysis		Second analysis	
	Storage period, days	mg/kg	Storage period, days	mg/kg
2	199	0.44	924	0.53
			924	0.42
4	190	0.5	915	0.5
			915	0.4
8	173	0.67	917	0.61
			922	0.52
6	156	0.79	876	1

In another study carried out in Japan, untreated apple samples were fortified with a solution of tebufenozide and its metabolites RH-6595 and RH-1788 at a level of 1 mg/kg. Samples were stored at -20ºC for 190-202 days. The average recoveries after storage were 94% for tebufenozide, 87% for RH-1788 and 69% for RH-6595 (Komatsu and Yabusaki, 1994).

The stability of residues of tebufenozide in apple juice during frozen storage was investigated by Deakyne and Chen (1995b). Samples of control apple juice (20 g) were fortified with 20 ì g of tebufenozide (1 mg/kg) and stored frozen. Samples were removed from storage at intervals and analysed together with control samples stored under the same conditions and freshly fortified. After 6 months storage the mean recovery was 86.7%

Grapes and wine. The stability of tebufenozide in frozen grapes and wine stored for periods up to 12 months was investigated by Schuld and Holzwarth (1994b). Samples were fortified with tebufenozide at 0.2 mg/kg (grapes) or 0.1 mg/kg (wine) and stored at ≤-18ºC. Samples were removed for analysis after approximately 0, 1, 3, 6, 9, and 12 months. There was no loss during the 12 months.

	% recovery of tebufenozide after period, months					
	0	1	3	6	9	12
Grapes		91	82.5	88	84	89
Wine	97	93	118	74	92	94.5

Walnuts. Walnut kernels were fortified with 1 mg/kg of tebufenozide and stored frozen at -10ºC (Cui and Deakyne, 1994). Controls, freshly fortified controls, and stored samples were analysed after 0, 1, 2, and 3 months storage. No decrease was observed during this period and the study was continued for a total of 18 months. The final residue was 76.6% of the initial level, showing that residues of tebufenozide are adequately stable for at least 18 months of frozen storage.

Lettuce. Control samples of lettuce were homogenized and sub-samples of 20 g each were fortified with 20 ì g of tebufenozide in methanol. After evaporation of the methanol the samples were sealed and stored frozen. Samples were removed from storage and analysed at intervals (Chen *et al.,* 1994b). After 128 days the mean recovery was 90%.

Rice. Samples of milled rice spiked with tebufenozide at 2 mg/kg were stored at -20ºC for 20-21 days until analysis. Recoveries ranged from 53 to 80% (Komatsu andYabusaki, 1992c).

The Meeting was informed that the studies on apples, walnuts and lettuce would be continued for a full 3 years of storage.

Residue definition

Tebufenozide was the predominant residue found in all the plant metabolism studies, which included apples, grapes, sugar beet and rice. Although metatbolism occurs in plants no single metabolite in any plant metabolism study exceeded 10% of the total residue.

In some residue trials samples were analysed for residues of the metabolites RH-6595 and RH-1788. The levels of these were very low, and in most cases below the limit of determination. Some samples in supervised trials on vegetables in the USA (1991-93) were analysed for the terminal metabolite RH-0897 (Chen *et al.,* 1994c,d). All residues were below the LOD.

The Meeting concluded that the residue should be defined as tebufenozide. The residue is fat-soluble.

USE PATTERN

Tebufenozide is an insectside which acts as an agonist of the critically important insect-moulting hormone, 20-hydroxyecdysone. Treatment with tebufenozide results in an accelerated, incomplete moult and an inmediate cessation of feeding in lepidopteran larvae.

Tebufenozide is used to control caterpillar pests in fruits, vegetables and other crops. The registered uses are shown in Table 20, and proposed uses in Table 21.

Table 20. Registered uses of tebufenozide.

Crop	Country	Form.	Application			PHI, days
			No.	Rate, kg ai/ha	Spray concn., kg ai/hl	
Apples	Canada	2SC	1-4	0.12-0.24		14
Apples	France	2SC	3	0.144	0.0144	21
Apples	Japan	20SC	2	0.4	0.0133	45
Apples	Korea	20SC	3	0.6		30
Apples	Switzerland	2SC	2-3	0.144-0.24	0.012	21
Cabbage species	Switzerland	2SC	1-2	0.096	0.0096	14
Chinese Kale	Thailand	20SC	3-5	0.1875	0.0333	14
Cotton	Colombia	2SC		0.036-0.060		
Grapes	France	2SC	3	0.144	0.0144	21
Grapes	Italy	2SC	1-3	0.144	0.0144	30
Grapes	Portugal	2SC	2-3	0.144	0.0144	Pending
Grapes	Spain	2SC	1-4	0.12-0.144	0.012-0.0144	21
Grapes	Switzerland	2SC	3-4	0.072-0.230	0.012-0.014	
Grapes	Thailand	20SC	2-3	0.1	0.00533	14

Crop	Country	Form.	Application			PHI, days
			No.	Rate, kg ai/ha	Spray concn., kg ai/hl	
Grapes	Turkey	2SC	1-3	0.096	0.0096	21
Kiwifruit	New Zealand	70WP	2-4	>0.12	0.006	21
Legumes	Vietnam	20SC	2-4	0.1		
Onions	Vietnam	20SC	2-4	0.1		
Onions, Welsh	Taiwan	20SC	3	0.1		9
Ornamental & Forestry Trees	Italy	2SC	2-3		0.0048-0.0096	
Peppers, Chilli	Indonesia	10WP	2-3	0.05-0.10		15
Peppers, Chilli	Indonesia	2SC	2-3	0.05-0.10		15
Pome fruit	Belgium	2SC	3	0.12	0.012	28
Pome fruit	Chile	2SC	1-4	0.24-0.36	0.0096	28
Pome fruit	Italy	2SC	1-4	0.216-0.288	0.0144-0.0192	14
Pome fruit	New Zealand	70WP	4-9	>0.18	0.006	14
Pome fruit	Portugal	2SC	2-3	0.144	0.0144	Pending
Rice	Colombia	2SC		0.036-0.060		
Rice	Japan	0.75DL	2	0.3		14
Rice	Japan	10WP	2	0.1-0.25		21
Rice	Korea	0.1G	3	0.3-0.5		3
Rice	Korea	8WP	6	0.08-0.12		3
Rice	Malasya	20SC	2	0.1		21
Rice	Philippines	20SC	1-2	0.1		14
Rice	Thailand	20SC	1-2	0.075	0.015	14
Shallots	Indonesia	20SC	4-6	0.075-0.15		15
Shallots	Indonesia	10WP	4-6	0.075-0.15		15
Soya beans	Indenesia	20SC	1-2	0.05-0.10		15
Soya beans	Indonesia	10WP	1-2	0.05-0.10		15
Sugar beet	Japan	20SC	2-4	0.1	0.01	14
Tea	Japan	20SC	2	0.4	0.02	14
Tea	Sri Lanka	20SC	1-2	0.13-0.15		7
Walnuts	France	2SC	3	0.144	0.0288	30
Walnuts	USA	2SC	4	0.28	0.0265	30

Table 21. Anticipated registered uses of tebufenozide.

Country	Crop	Form.	Application			PHI, days
			No	Rate, kg ai/ha	Spray conc., kg ai/hl	
Australia	Pome fruit	70WP, 2SC	4-8	0.18	0.006	28
	Grapes	70WP, 2SC	1-3	0.06	0.006	28
Austria	Pome fruit	2SC	2-3	0.09-0.18	0.012	14
	Grapes	2SC	3-4	0.072-0.192	0.012	28
Cyprus	Pome fruit	2SC	2	0.12	0.012	14
	Citrus fruit	2SC	1-2	0.12-0.24	0.012-0.024	14
France	Pears	2SC	3	0.144	0.0144	21
Germany	Pome fruit	2SC	2-3	0.09-0.18	0.012	14
	Grapes	2SC	3-4	0.072-0.192	0.012	28
Greece	Pome fruit	2SC	4	0.288	0.0144	14
	Grapes	2SC	4	0.144	0.0144	21
Slovenia	Apples	2SC	1-3	0.216	0.0144	21
	Grapes	2SC	1-3	0.12	0.012	21

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Country	Crop	Form.	Application			PHI, days
			No	Rate, kg ai/ha	Spray conc., kg ai/hl	
Spain	Pome fruit	2SC	1-3	0.15-0.18	0.012-0.014	21
	Rice	240LV	2-3	0.12		21
Switzerland	Lettuce spinach	2SC	1-2	0.15-0.18	0.0096	14
USA	Pome fruit	2SC, 70WP	6	0.35		14
	Grapes	2SC		not for registration	filed import tolerance	
	Kiwifruit	70WP	(2-4)	(0.134)	(>0.12)	90
	Pecans	2SC	5	0.28	0.0347	14
	Cole crops	2SC, 70WP	7	0.067-0.135		7
	Leafy vegetables	2SC, 70WP	7	0.067-0.135		7
	Cotton	2SC	4	0.28		14