DIFLUBENZURON (130)

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EXPLANATION

Diflubenzuron, an insecticide, which was originally evaluated by the JMPR in 1981 and re-evaluated for residues several times up to 1988. At the 28th Session of the CCPR in 1996 (ALINORM 97/24) diflubenzuron was scheduled for the JMPR in 1999 as a priority compound under the Periodic Review Programme. However, the manufacturer asked for a postponement and therefore the periodic review of diflubenzuron was re-scheduled for the JMPR in 2002.

The basic manufacturer supplied information on identity, metabolism and environmental fate, residue analysis, use pattern, residues resulting from supervised trials on crops (almonds, apples, berries, blackcurrants, Brussels sprouts, chilli peppers, cotton, gooseberries, grapefruit, head cabbages, lemons, limes, mandarins, mushrooms, oranges, peaches, pears, peas, pecans, plums, range grass, rice, soya beans, sweet peppers, tomatoes, walnuts), fate of residues during storage or in processing, residues in animal commodities (meat, milk, eggs) resulting from direct animal treatment or feeding, and national MRLs. In addition, information on GAP and national MRLs was supplied by the governments of The Netherlands, Germany and Australia.

IDENTITY

ISO common name: diflubenzuron

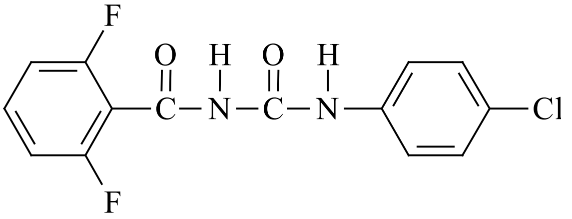
Chemical name
IUPAC: 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea
CA: *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide

CAS Registry No: 35367-38-5

CIPAC No: 339

Synonyms and code names: DU 112317, PH 60-40, TH-6040 [White, 1998]

Structural formula: (Borst, 1982)



Molecular formula: $C_{14}H_9ClF_2N_2O_2$

Molecular weight: 310.7

Physical and chemical properties

Pure active ingredient

(some properties refer to diflubenzuron of purity <99.5%, as indicated)

Minimum purity: 99.5% (w/w, analytical grade)

Appearance: colourless crystals

Vapour pressure: 1.2×10^{-7} Pa (9.3×10^{-10} mm Hg) at 25ºC
technical grade; OECD 104; (Harteveld, 1988)

Melting point: 501ºK, 228ºC; OECD 102 (Thus *et al.*, 1995)

Octanol/water partition coefficient, purity 97.6%, (Thus, 1988):
$\log K_{ow} = 3.89$ for diflubenzuron
$\log K_{ow} = 1.14$ for 4-chlorophenylurea (CPU)
$\log K_{ow} = -0.02$ for 2,6-difluorobenzoic acid (DFBA)

Solubility: OECD 105:
water 8×10^{-5} g/l at 25ºC (Balder *et al.*, 1989)
0.05 M phthalate buffer pH 4, 10×10^{-5} g/l at 25ºC
0.05 M phosphate buffer pH 7, 8×10^{-5} g/l at 25ºC
0.05 M borate buffer pH 10, 32×10^{-5} g/l at 25ºC (Van Kempen *et al.,* 1995);
methanol 1.1 g/l at 20ºC
dichloromethane 1.8 g/l at 20ºC
n-hexane 0.063 g/l at 20ºC
toluene 0.29 g/l at 20ºC (Van Kempen and Feenstra-Bielders, 1995)
purity 99.1%; not according to OECD 105:
acetone 6.98 g/l at 20ºC
ethyl acetate 4.26 g/l at 20ºC (Yu, 1999a)

Relative density: 1.569 g/cm^3 at 20ºC; OECD 109, (Hengelmolen, 2000)

Hydrolysis: non-GLP, EPA guideline 161-1, [^{14}C]diflubenzuron labelled in both phenyl rings, radiochemical purity 99.2%, specific activity 544 MBq/g, chemical purity 97.1%; in the dark at 25 ± 1°C (Boelhouwers *et al.*, 1988b; De Boer, 1989)
pH 5, stable (<10% transformation after 4 weeks)
pH 7, stable (<10% transformation after 4 weeks)
pH 9, half-life 32 days (46% transformation after 4 weeks, degradation compounds 26% CPU, 15% DFBA, 1% PCA, 1% DFBAM, 3% missing; identification by mass spectrometry).

purity unknown, in the dark (De Wilde, 1985)
pH 5 stable (2% transformation after 150 days 22°C or 11-23% transformation after 63 days 20°C)
pH 7 stable (0% transformation after 150 days 22°C or 27-31% transformation after 63 days 20°C)
pH 9 half-life 44 days (22°C) or 40-42 days (20°C)
pH 12 half-life 7.5 days (20°C)

Photolysis:	solid, purity 99.1% (Friedlander, 1999a), no significant decomposition (<0.004%) after exposure to simulated sunlight for 7 days at 20ºC in phosphate buffer pH 7 plus 1% acetonitrile, purity 97.1%, (Boelhouwers *et al.*, 1988a), half life 32-47 days after exposure to 450 W Xenon arc (≥290 nm) for 15 days at 25ºC (equivalent to 30 days sunlight)
Dissociation constant:	not measurable owing to low water solubility (Yu, 1999b)

Technical material

Minimum purity:	97.9% w/w (Van Kampen and Thus, 1996)
Main impurities:	no information
Appearance:	white, non-volatile, dusty powder at 20°C, with a faint odour characteristic of aromatic compounds, purity 99.1% (Friedlander, 1998, 1999b,c)
Relative density:	1.41 g/cm^3 ± 3.43% (RSD, n=3), technical purity, (Stevenson, 1998)
Melting range:	no information
Stability:	1.1% decomposition in a simulated long-term stability test 14 days at 54ºC at a pressure of 25 g/cm^2; purity 99.1% (Riggs, 1999).

Formulations

The following formulations (g ai/kg or g ai/l) were identified from the information supplied by the manufacturer: BR (briquette; 10 g/kg); DP (dustable powder; 3.75 g/kg), GR (granules; 20 g/kg; 40 g/kg), OF (oil miscible flowable concentrate 450 g/l; 60 g/l), SC (suspension concentrate; 50 g/l; 150 g/l; 240 g/l; 480 g/l), SU (ultra-low volume suspension; 9 g/l; 15 g/l), WG (water dispersible granule; 800 g/kg) and WP (wettable powder; 50 g/kg; 100 g/kg; 250 g/kg). The product is also used in combination with other pesticides (40 g/kg plus 192 g/kg methomyl or 33 g/kg plus 180 g/kg methidathion), as a feed-through product (100 g/kg) and as a veterinary drug for salmon (Lepsidon 0.6 g/kg), cattle (Stampede 20 g/l) and sheep (Fleececare 250 g/l; Strike 250 g/l, IGR jet and dip (concentration not reported), Magnum IGR 25 g/l, Duodip (concentration not reported)).

Abbreviations

The following abbreviations are used in addition to those previously listed.

ACN	acetonitrile
APCI	atmospheric pressure chemical ionisation (for MS detection)
CEC	cation exchange capacity
CFU	colony forming unit
CPHFBA	*N*-(4-chlorophenyl)heptafluorobutanamide or 4-chlorophenyl-*N*-(heptafluorobutyryl)-anilide (derivatization product of CPU or PCA with HFBA)
CPU	4-chlorophenylurea; *p*-chlorophenylurea
DAT	days after last treatment
DFBA	2,6-difluorobenzoic acid
DFBAM	2,6-difluorobenzamide
DFHA	2,6-difluorohippuric acid; glycine conjugate of DFBA

DMSO	dimethyl sulfoxide
DL	detection limit
DT-50	period required for 50% dissipation: 50% of the parent has disappeared. Also called half-life.
DT-90	period required for 90% dissipation: 90% of the parent has disappeared
2D-TLC	two dimensional thin layer chromatography
GC-ID-MS	gas chromatography with isotope dilution and mass spectrometric detection
GC-ECD	gas chromatography with electron capture detection
GC-FID	gas chromatography with flame ionisation detection
GPC	gel permeation chromatography
HFBA	heptafluorobutyric acid anhydride (derivatization agent for CPU and PCA)
HPLC-LSC	high performance liquid chromatography with detection by liquid scintillation counting
HPLC-UV	high performance liquid chromatography with spectrophotometric detection at ultra violet wavelengths
HV	high volume spray
K_F	Freundlich constant
K_{om}	organic matter adsorption coefficient
$K_{s/l}$	solid-liquid partition coefficient (l/kg)
LOQ	limit of quantification = limit of quantitation = limit of determination
LSC	liquid scintillation counting of radioactivity
LV	low volume spray
2-OH-DFB	1-(4-chloro-2-hydroxyphenyl)-3-(2,6-difluorobenzoyl) urea; *N*-[[(4-chloro-2-hydroxyphenyl)amino]carbonyl]-2,6-difluorobenzamide; 4-chloro-2-hydroxydiflubenzuron
3-OH-DFB	1-(3-hydroxy-4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea; *N*-[[(4-chloro-3-hydroxyphenyl)amino]carbonyl]-2,6-difluorobenzamide; 4-chloro-3-hydroxydiflubenzuron
3-OH(F)-DFB	*N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluoro-3-hydroxybenzamide; 2,6-difluoro-3-hydroxydiflubenzuron
om	organic matter content of soil
PCA	4-chloroaniline; *p*-chloroaniline
PCAA	4-chloroacetanilide; *p*-chloroacetanilide
PF	moisture content of soil
RSD_r	precision under repeatability conditions (measurements within one day or one run) expressed as relative standard deviation (= coefficient of variation)
RSD_R	precision under within-laboratory (intra-laboratory) reproducibility conditions (measurements covering several days or runs within the same laboratory) expressed as relative standard deviation (= coefficient of variation). This value indicates precision under varying conditions.
THF	tetrahydrofuran
TRR	total recovered radioactivity in specified part of plant or animal
ULV	ultra low volume

METABOLISM AND ENVIRONMENTAL FATE

Animal metabolism

The Meeting received information on the fate of orally dosed diflubenzuron in lactating cows, male sheep, lactating goats, laying hens, and pigs, of diflubenzuron applied dermally to cattle, and of diflubenzuron when incubated with fluids from the rumen, abomasum and small intestine of cattle and sheep. Studies on laboratory animal metabolism (rat, mouse, rabbit, cat) were evaluated by the WHO Core Assessment Group at the 2001 JMPR.

Cattle. The distribution of radioactivity resulting after repeated oral administration of [^{14}C]diflubenzuron (equally labelled in both phenyl moieties; specific activity 17.42 mCi/mmol; radiochemical purity not reported) was studied in lactating cows receiving daily doses in gelatine capsules over a 28-day period (Smith and Merricks, 1976a, non-GLP). Twenty-two Holstein dairy cows (431-544 kg) were dosed with 0 (1 cow), 0.05, 0.5, 5.0, 25 (4 cows each) and 250 ppm in the feed (5 cows). The authors do not state whether doses were expressed on the dry weight. Since the cows were fed 11 kg feed per day, this corresponds to dose levels of about 0.001, 0.01, 0.1, 0.5 and 5 mg ai/kg bw per day. The 0.05, 0.5 and 5.0 ppm doses were radioactive, with specific activities of 1.63, 0.36 and 0.29 mCi/mmol respectively. The 25 ppm dose was unlabelled, as were four of the five 250 ppm doses. One cow received a radiolabelled 250 ppm dose, with a specific activity of 0.034 mCi/mmol. Cows in each group were slaughtered at day 1, 18 or 28 of dosing or after a 7- or 14-day withdrawal period. Milk samples were taken every 4 days from cows exposed for 28 days, and on days 2, 4, 5, 6, and 7 from the radiolabelled high-dose cow, which was slaughtered on day 8. Samples were stored frozen (temperature and storage times not reported). Round muscle, loin muscle, fat, liver, kidney and milk were analysed for ^{14}C by combustion and LSC. Analyses of non-radioactive tissues were carried out by HPLC-UV method AM-3 described below (Residue analysis).

No information was given on the excretion routes of the total radiolabel. No residues in milk were detected at the 0.05 (DL 0.0003 mg/kg) or 0.5 ppm dose levels (DL 0.001 mg/kg). An average of 0.0091 mg/kg diflubenzuron equivalents was found in the milk from the 5.0 ppm dose level: a plateau was reached between day 4 and day 7. After 4 days of withdrawal, residues in milk were undetectable (DL 0.002 mg/kg). At the 250 ppm dose level, a plateau was reached in milk by day 2 at 0.20 mg/kg diflubenzuron equivalents. Milk was acidified (pH 2) and extracted with ethyl acetate, followed by clean-up and analysis by TLC. The radioactivity in the extract (61%-72% of the TRR in milk) was not due to diflubenzuron itself, but to unspecified metabolites.

Total ^{14}C analyses showed no residues in muscle, fat, kidney or blood at dose levels of 0.05, 0.5 and 5 ppm (DL 0.007, 0.03, and 0.04 mg/kg respectively). Only the liver contained dose-related residues, where a plateau was reached by day 18 for all radioactive doses. Withdrawal for 7 days did not result in a substantial decrease in activity. Radiocarbon residues in liver were 0.0084 mg/kg (days 18, 28) at the 0.05 ppm level, 0.077 mg/kg at the 0.5 ppm level, and 0.54 mg/kg at the 5 ppm level. At the 250 ppm dose level, only tested for 7 days of treatment, residues were found in the kidney and liver (1.0 and 6.0 mg/kg diflubenzuron equivalents) but not in muscle or fat (DL 0.04 mg/kg).

HPLC analysis at the 250 ppm dose level yielded diflubenzuron levels of 0.08 mg/kg in fat, 0.10 mg/kg in liver, and <0.05 mg/kg (LOQ) in milk, muscle and kidney. The ^{14}C and HPLC analyses of liver (6.0 mg/kg diflubenzuron equivalents and 0.10 mg/kg diflubenzuron) are in good agreement, since 19% of the ^{14}C in the liver was extractable and 9.8% of this was diflubenzuron. This corresponds to 0.11 mg/kg diflubenzuron by ^{14}C measurement.

Liver from the cow at the 250 ppm dose level (about 5 mg/kg bw per day) was extracted using various solvents. Extractabilities were 19% for ACN, 21% for ethyl acetate, 24% for methylene chloride, 25% for acetone, 31% for chloroform/methanol (1 + 2), 32% for 3 M HCl/methanol (1 + 9) and 32% for 7.5% anhydrous HCl in methanol. Hydrolysis after 15 hours of reflux with 1 M or 6 M HCl caused degradation of diflubenzuron. Enzymatic hydrolysis for 15 hours at 37°C with glucuronidase, sulphatase or protease did not release any additional ethyl acetate extractable radioactivity.

Ethyl acetate extracts were further purified either by silica gel or celite partition column chromatography. The fractions containing radioactivity were analysed by TLC. Diflubenzuron, CPU, PCA and DFBA were used as unlabelled reference compounds. The identity of the parent compound was confirmed by MS.

TLC analysis of the silica gel-purified fractions of the ethyl acetate extracts (26% of the TRR in liver), showed that the two radioactive components found were DFBA (20% of the TRR in liver = 1.2 mg/kg diflubenzuron equivalents) and the parent (5.9% of the TRR in liver = 0.36 mg/kg). Analysis of the celite-purified fractions of the extracts more or less confirmed this distribution, since DFBA accounted for 13% of the TRR in liver (0.81 mg/kg diflubenzuron equivalents) and the parent for 3.7% (0.22 mg/kg). However, the celite-purified fractions contained other metabolites: CPU accounted for 0.2% of the TRR (0.12 mg/kg diflubenzuron equivalents), PCA for 1.4% (0.085 mg/kg diflubenzuron equivalents) and unknown extractable metabolites for 7.6% (0.46 mg/kg).

A 360 kg lactating Jersey cow received a single oral dose of [^{14}C]diflubenzuron (equally labelled in both phenyl moieties, specific activity 17.4 mCi/mmol, radiochemical purity >99.9%, 10 mg ai/kg bw) as a slurry in water by stomach tube (Ivie, 1978, non-GLP). Total urine and faeces samples were collected at 24-hour intervals, and the animal was machine-milked every 12 hours. Seven days after treatment, the cow was slaughtered and samples of liver, kidney, muscle and fat were collected and stored frozen (temperature and storage time not reported). The radiocarbon content of the tissues, milk, urine, faeces and blood was determined by combustion and LSC. Urine, milk and faeces samples were acidified to pH 2 and extracted with ethyl acetate followed by clean-up (if necessary). Metabolites in extracts of milk, urine and faeces were resolved by 2D-TLC, and identified by comparison with reference compounds (CPU, DFBA, DFBAM, PCA, 2-OH-DFB, 3-OH-DFB, PCAA, 4-chlorophenol, 4-chloro-*N*-methylaniline and 4-chloro-*N,N*-dimethylaniline), followed by MS or NMR analysis.

The main metabolite 3-OH(F)-DFB was isolated from urine and faeces by preparative TLC and administered to two 275 g adult female Sprague-Dawley rats (15 mg/kg bw, single dose) by stomach tube. Urine and faeces samples were collected daily for 3 days after treatment. The rats were then killed and 21 different tissue samples were isolated. Faeces, urine and tissues were analysed for radiocarbon content and the nature of the excreted radioactivity was investigated by 2D-TLC.

During the 7-day post-treatment period about 85% of the administered ^{14}C was eliminated in the faeces, about 15% in the urine and about 0.2% was secreted into the milk. There was little tendency to retain ^{14}C in the body tissues. The maximum milk residues were 0.8 mg/kg diflubenzuron equivalents, which appeared in the sample collected 24 hours after treatment. Radiocarbon in the milk dropped to undetectable levels (DL 0.1 mg/kg) 3 days after treatment. No residues were found in tissues (DL 0.1 mg/kg), except liver (2.9 mg/kg diflubenzuron equivalents).

Metabolites found in ethyl acetate extracts of faeces, urine and milk are shown in Table 1. Residues in liver were not further investigated. Incubation of water-soluble metabolites with β-glucuronidase-arylsulphatase indicated that half of the water-soluble radiocarbon in urine was in glucuronide or sulfate conjugates. The major metabolic transformation was hydroxylation at the 3- or 4-position of the 2,6-difluorobenzoyl ring. Other identified metabolic pathways were cleavage between the carbonyl and amino groups to give DFBA, DFBAM, PCA and CPU. PCAA, a presumed acetylation product of PCA, was also tentatively identified in urine, faeces and milk.

Administration of the major hydroxylated diflubenzuron metabolite (3-OH(F)-DFB) to rats resulted in rapid (<3 days after administration) and quantitative excretion (71% via faeces, 23% via urine) with little further biotransformation.

Table 1. Metabolites in faeces, urine and milk from a lactating cow after a single oral treatment with 10 mg/kg bw [^{14}C]diflubenzuron.

Compound	% of TRR		
	Faeces	Urine	Milk
diflubenzuron [1]	42	-	43
3-OH(F)-DFB [1]	18	45	12
2-OH-DFB [1]	0.6	1.6	

Compound	% of TRR		
	Faeces	Urine	Milk
3-OH-DFB [1]	0.7	3.7	
CPU		0.6	
DFBA [1]		6.0	
DFHA [1]		6.9	1.9
DFBAM			13
unknowns in ethyl acetate extract	9.3[2]	19[3]	13[4]
water-soluble		18[5]	18
unextractable	29		
total	100	100	100

[1] molecular structure confirmed by NMR or MS
[2] contains 4 unknown metabolites (0.8%; 1.1%, 2.1%, 5.3% of TRR)
[3] contains 2 unknown metabolites (5.6% and 13% of TRR)
[4] contains 4 unknown metabolites (0.4%, 1.6%, 2.5%, 8.1% of TRR)
[5] when hydrolysed with β-glucuronidase-arylsulphatase, 7 unknown metabolites were found in ethyl acetate extracts

To evaluate the persistence of diflubenzuron on the hair and skin of cattle, 20 x 20 cm areas were marked off on each side of three Black Angus steers weighing 300-400 kg (Ivie, 1978, non-GLP). The hair immediately surrounding the treatment area was closely clipped, but hair on the treatment area was not disturbed. The 400 cm^2 areas were then treated with either a 1% ai WP or 1% ai oil-based formulation, both containing [^{14}C]diflubenzuron as a tracer (equally labelled in both phenyl moieties, specific activity 17.4 mCi/mmol, radiochemical purity >99.9%). The amount of radiocarbon applied to each of the treatment areas was determined by reweighing the treatment solution. In each case the applied [^{14}C]diflubenzuron was equivalent to about 0.125 mg/cm^2 of hide. The total volume of liquid applied to each area was about 5 ml. After treatment, the animals were transferred to a 2-ha pasture that was not protected from the elements except by afternoon shading. At 1, 2 and 4 weeks, one animal was killed and the treated skin patches were removed for measurement of residual radiocarbon. In addition, a number of tissue samples (not specified) were taken for combustion analysis.

[^{14}C]diflubenzuron residues were rapidly dissipated after dermal application (Table 2). The 4-week study was conducted during late summer, a period of mostly sunny and hot weather. A total of about 2 cm of rain was recorded during the study period, but rain clearly was not the cause of the rapid dissipation. The first rain did not fall until 1 day after the 1-week animal was killed. TLC analysis of acetone rinses of the treated areas showed that diflubenzuron was the only radioactive compound present in all samples. Analysis of numerous tissues taken when the animals were killed showed none, except hair and skin samples collected at or adjacent to the treated areas, to have detectable radiocarbon residues (DL not reported).

Table 2. Persistence of [^{14}C]diflubenzuron on hair and skin after dermal application to cattle held in an unprotected pasture.

Weeks after treatment	Residual radiocarbon, % of applied	Hair, mg/kg diflubenzuron equivalents	Skin, mg/kg diflubenzuron equivalents
WP formulation			
1	3.8	85	0.4
2	1.7	20	0.1
4	0.1	2.9	<0.1
Oil-based formulation			
1	3.5	128	0.4
2	0.7	20	0.1
4	0.1	3.8	<0.1

In a second study (same reference), designed to determine whether diflubenzuron is absorbed through the skin after dermal exposure, a 525-kg cow was stanchioned indoors and catheterised, and two 20 x 20 cm areas were marked off and treated as before, except that only the WP formulation was used. During 3 days after treatment, total urine and faeces samples were collected at 24-hour intervals and analysed for radiocarbon. After 3 days the cow was not killed but the treated areas were clipped closely and the exposed skin cleaned thoroughly with tissue paper moistened with acetone. The radiocarbon on the hair and paper was then quantified and extracted, and the extract analysed by TLC.

The studies indicated that diflubenzuron applied as WP is not absorbed through the skin to any significant extent after dermal application. During a 3-day period after application, no detectable residues were excreted in the urine (DL not reported). Although 2.1% of the applied radiocarbon was found in the faeces, the author strongly suggests that this represents contamination by rub-off or other external transfer processes, since the faeces were allowed to remain where deposited until collected at 24-hour intervals. After 3 days, 68% of the applied radioactivity was recovered by clipping and extracting the treated hair and thoroughly washing the exposed skin with acetone. TLC of these fractions showed diflubenzuron as the only radioactive compound.

In the same series of experiments rumen fluid was obtained by stomach tube from a 450-kg steer. Aliquots of the fluid (10 ml) were incubated for 24 hours at 39-40°C with 0.003 mM [^{14}C]diflubenzuron (equally labelled in both phenyl moieties, specific activity 17.4 mCi/mmol, radiochemical purity >99.9%). [^{14}C]diflubenzuron was not degraded to any significant extent when incubated in vitro with digestive tract fluids of cattle.

Sheep. The common bile ducts of two of four-mixed breed castrated male sheep (28-42 kg) were surgically cannulated at least 7 days before [^{14}C]diflubenzuron treatment (Ivie, 1978). One cannulated and one uncannulated sheep were given single oral doses of [^{14}C]diflubenzuron (equally labelled in both phenyl moieties, specific activity 17.4 mCi/mmol, radiochemical purity >99.9%, 10 mg ai/kg bw) as a slurry in water by stomach tube. For easier isolation of larger quantities of [^{14}C]diflubenzuron metabolites, the other two sheep (one cannulated and one uncannulated) were treated orally with [^{14}C]diflubenzuron at 500 mg ai/kg bw. Total urine, bile, and faeces samples were collected at 24-hour intervals. Four days after treatment the two sheep treated at 10 mg/kg bw were killed, and brain, liver, kidney, muscle and fat were collected. Samples were stored frozen (temperature and storage time not reported). Urine, bile, faeces and tissues were radioassayed by combustion and LSC. Urine, bile and faeces samples were acidified to pH 2 and extracted with ethyl acetate followed by clean-up if necessary. Metabolites in extracts of urine, bile and faeces were resolved by 2D-TLC, and identified by comparison with reference compounds (CPU, DFBA, DFBAM, PCA, 2-OH-DFB, 3-OH-DFB, PCAA, 4-chlorophenol, 4-chloro-*N*-methylaniline and 4-chloro-*N,N*-dimethylaniline), followed by MS or NMR.

In the 4-day post treatment period, the uncannulated sheep treated at the 10 mg/kg bw level eliminated 42% of the administered ^{14}C in the faeces and 41% in the urine. The cannulated sheep at this dose eliminated 36% in the bile, 32% in the faeces, and 24% in the urine. In the same period, the uncannulated sheep treated at 500 mg/kg bw eliminated 79% in the faeces and 10% in the urine. The cannulated sheep at this high dose eliminated only 5% in the bile, 74% in the faeces, and 7% in the urine. There was little tendency to retain ^{14}C in the body tissues, so 8%-17% of the administered radioactivity was missing.

At the 10 mg/kg bw dose the liver of the uncannulated sheep was the only organ or tissue which contained radioactive residues (2.3 mg/kg diflubenzuron equivalents); the cannulated sheep had 3.6 mg/kg diflubenzuron equivalents in the liver and 0.40 mg/kg diflubenzuron equivalents in the kidney. Residues in other tissues were below the detection limit (0.05 mg/kg).

Metabolites found in faeces and urine are shown in Table 3. Residues found in the liver and kidneys were not further investigated. At least eight radioactive components were found in the bile extracts. Three of these were identified as the hydroxydiflubenzuron isomers seen in urine and faeces, but these metabolites collectively accounted for <5% of the total radiocarbon in the bile. The other metabolites remained unidentified. Incubation of water-soluble metabolites with β-glucuronidase-arylsulfatase indicated that half of the water-soluble radiocarbon in the urine and bile was in the form of glucuronide or sulfate conjugates.

Table 3. Metabolites in faeces and urine (% of TRR) from uncannulated and cannulated castrated male sheep after oral treatment (single dose) with 10 mg/kg bw [^{14}C]diflubenzuron.

Compound	% of TRR				
	Uncannulated		Cannulated		
	faeces	urine	faeces	urine	bile
diflubenzuron [1]	40		98		
3-OH(F)-DFB [1]	0.4	1.4		1.2	<5
2-OH-DFB [1]	0.8	0.2		0.3	
3-OH-DFB [1]	0.4			0.4	
DFBA [1]		27		15	
DFHA[1]		22		30	
unknown	6.0[2]	16[3]		24[3]	45-65
water-soluble		34[4]		29[4]	30-50[5]
unextractable	52		2.3		
total % of TRR	100	100	100	100	100
% of dose	42	41	32	24	36

[1] molecular structure confirmed by NMR or MS.

[2] contained 2 unknown metabolites (1.2% and 4.8% of TRR)

[3] contained 4 unknown metabolites (0.2-0.3%, 0.4%, 2.1-2.6% and 13-21% of TRR)

[4] when hydrolysed with β-glucuronidase-arylsulfatase, 6 unknown metabolites were found in ethyl acetate extracts and one metabolite (<5% of TRR) was identified as 3-OH(F)-DFB.

[5] when hydrolysed with β-glucuronidase-arylsulfatase, 8 unknown metabolites were found in ethyl acetate extracts

Fluids from the rumen, abomasum, and small intestine were collected from two pairs of mixed-breed ewes (same reference). Before being killed one pair was fed a high-energy sheep ration plus alfalfa hay, the other pair an all-roughage diet consisting solely of Coastal Bermuda grass hay, for 4 weeks. Aliquots of the fluids (10 ml) were incubated for 24 hours at 39-40°C with 0.003 mM [^{14}C]diflubenzuron (equally labelled in both phenyl moieties, specific activity 17.4 mCi/mmol, radiochemical purity >99.9%). The diflubenzuron was not degraded to any significant extent.

Goats. Two groups of two lactating goats (British Sannen; 33-71 kg) were dosed by gavage twice daily for 3 days with radiolabelled [^{14}C]diflubenzuron (equally labelled in both phenyl moieties; radiochemical purity >99%) at 0.1 or 2.5 mg ai/kg bw/dose with a specific activity of 0.722 and 0.037 MBq/mg for the low and high doses respectively (Cameron *et al.*, 1991, GLP). There was one control. Excreta and milk were collected before each dose. At 15 hours after the last dose, the animals were slaughtered and liver, kidney, muscle and subcutaneous fat were sampled and stored at –20°C (storage time not reported). Total radioactivity was determined by combustion and LSC.

In both dose groups radioactivity was mainly excreted in the faeces (Table 4). Urinary excretion of total radioactivity up to 15 hours after the last dose was a marginally lower proportion of the higher dose representing 3.9-8.1% of the dose compared with 11-14% of the dose at the lower level. Milk residues reached a plateau over the first 8 hours at the low dose level and within 8-24 hours at the high level. Only about 0.1% of the total administered dose was recovered in milk. Residue levels in tissues are shown in Table 4. The levels of radioactivity in muscle and fat were below the LOQ (<30 dpm above background) for both dose groups.

Table 4. Percentage radioactivity and mg/kg diflubenzuron equivalents in excreta and tissues from lactating goats following repeated oral treatment with either 0.1 or 2.5 mg/kg bw/dose of [^{14}C]diflubenzuron.

Sample	0.1 mg/kg bw/dose		2.5 mg/kg bw/dose	
	mg/kg as diflubenzuron	% of total dose	mg/kg as diflubenzuron	% of total dose
Excreta + milk collected over total duration of exposure				
Faeces		73-81		76-86
Urine		11-14		3.9-8.1
Cage wash		1.8-2.0		1.0-1.9
Milk	0.004-0.009	0.09-0.10	0.12-0.22	0.07-0.11
Tissues + intestinal contents at slaughter				
Intestinal contents	0.17-0.19	7.7-9.0	4.0-7.5	15
Liver	0.22-0.26	0.73-0.83	3.2-6.1	0.42-0.72
Bile	0.15-0.23	0.01	2.6-21	nq-0.07
Kidney	0.016-0.019	0.01	0.36-1.0	0.01-0.02
Intestinal wall	0.018-0.021	0.25-0.29	0.39-2.0	0.21-1.1
Carcase	0.006-0.008	0.46-0.60	0.12-0.18	0.34-0.53
Total		99-103		102-107

nq: below LOQ (<30 dpm above background)

Radioactive residues in the liver and milk samples were characterized and identified (Dijksman *et al.*, 1990; Timmerman *et al.*, 1992a-c; Timmerman, 1997; all GLP). All samples were stored homogenized below –10°C (storage time 22-24 months for liver and 1-2 months for milk).

Liver samples were extracted with several solvents. Extraction efficiencies from liver were 12% of the TRR for ethyl acetate; 28% for 10% acetic acid; 33% for 5% ammonia followed by ACN/ethyl acetate (1 + 1); 41% for tetrahydrofuran; 45% for dioxane/ACN (5 + 2); 46% for water; 49% for DMSO, 50%-77% for skimmed milk, 61% for butanol, 65% for dimethylformamide; 65% for calf serum, and 105% for 10% ammonia. Because of the high extraction efficiency 10% ammonia was chosen as extraction solvent; it extracted 87% of the TRR from milk. According to the authors no extraction artefacts were introduced because no qualitative differences were observed in extracts with skimmed milk and with 10% ammonia. Ammonia extracts were fractionated by HPLC (concentration on RP-8 pre-column, separation on C-8 column, two solvent systems) with off-line LSC detection. Reference compounds used for liver were diflubenzuron, DFBA, DFHA, DFBAM, PCA, CPU, 2-OH-DFB, 3-OH-DFB, 4-chloronitrobenzene and *N*-acetyl-4-chlorophenylurea. Reference compounds used for milk were diflubenzuron, CPU, PCA and PCAA. Because of its suspected toxic properties PCA was also determined in liver and milk by GC-ECD method I (described in Residue analysis).

The HPLC metabolite patterns from liver extracts showed more than 15 peaks ranging from 0.0020 to 0.033 mg/kg as diflubenzuron for the low-dose and 0.029 to 0.91 mg/kg for the high-dose group (Table 5). Enzymatic hydrolysis (with β-glucuronidase, arylsulfatase and sulfatase) did not change the patterns. About 14% of the TRR in the livers of goats of the low- and high-dose groups consisted of CPU. Low levels of PCA (0.011 and 0.028 mg/kg) were found by GC-ECD analysis in liver samples of the two goats of the 2.5 mg/kg bw/dose group but not the 0.1 mg/kg group (<LOQ of 0.01 mg/kg).

HPLC analysis showed that the residue in milk consisted of about 8 components. Attempts to identify them failed. Enzymatic hydrolysis showed that 20% of the metabolites were conjugates, about 13% being sulfate(s) and 7% glucuronide(s). No PCA was found above the LOQ (0.001 mg/kg) by GC-ECD analysis.

Since the storage stability of diflubenzuron, CPU, PCA and PCAA in the goat liver and milk samples showed a high variability (RSD>20%, see section on storage stability) the interpretation of the results from this study is rather difficult.

Table 5. Metabolite pattern expressed as % of the TRR and mg/kg diflubenzuron equivalents (in parenthesis) in ammonia extracts of the liver of lactating goats following repeated oral treatment with either 0.1 or 2.5 mg/kg bw/dose [^{14}C]diflubenzuron.

Compound or fraction	0.1 mg/kg bw/dose		2.5 mg/kg bw/dose	
	goat 1	goat 3	goat 4	goat 5
TRR	0.22 mg/kg	0.26 mg/kg	0.0032 mg/kg	0.0061 mg/kg
CPU	16% (0.033)	11% (0.027)	13% (0.39)	16% (0.91)
DFBAM	4.0% (0.0081)	5.3% (0.013)	1.5% (0.064)	1.4% (0.076)
diflubenzuron	4.1% (0.0081)	3.5% (0.0043)	7.0% (0.22)	3.7% (0.21)
unknown	56%[1]	71%[1]	55%[2]	60%[2]
total[3]	81%	91%	77%	81%

[1] 11-12 unknown metabolites (1.0%-12% of TRR, corresponding to 0.0020-0.024 mg/kg diflubenzuron equivalents)

[2] 14 unknown metabolites (1.3%-11% of TRR, corresponding to 0.029-0.37 mg/kg diflubenzuron equivalents)

[3] the missing radioactivity was retained on the HPLC pre-column.

A later report based on the same data (Ruijten, 1994, GLP) concluded that no PCA was found in the liver of the 2.5 mg/kg bw/dose group since the levels were below the LOQ (0.03 mg/kg), i.e. too low to reliably assess the presence of PCA. In view of the storage stability results this is uncertain.

Hens. Eight adult laying hens, 4 White Leghorn (WL), 36 weeks old, about 1500 g, and 4 Rhode Island Red/Barred Plymouth Rock Buff (RIR/BPR), 46 weeks old, about 2600 g, received single oral doses by capsule of approximately 5 mg/kg bw [^{14}C]diflubenzuron (equally labelled in both phenyl moieties; specific activity 17.42 mCi/mmol, radiochemical purity >99%), which is about 10 times the dose needed to control housefly larvae in the excreta (Opdycke, 1976; Opdycke *et al.*, 1976, 1982b, all non-GLP). Excreta from individual hens were collected at specified intervals for 12 days (WL) or 13 days (RIR/BPR). Eggs were collected at 12-hour intervals for 11 and 10 days respectively. The WL and RIR/BPR hens were killed after 12 and 13 days respectively, and samples of tissues were taken for ^{14}C measurement. Samples were stored at –20°C (time not reported), then extracted with ethyl acetate. Unextracted ^{14}C was determined by combusting in an oxidizer. All results were expressed as mg/kg diflubenzuron based on the original wet weight. Metabolites purified from the excreta were characterized by TLC co-chromatography and HPLC with the reference compounds CPU, PCA, DFBA, 2-OH-DFB and 3-OH-DFB.

91% of the administered dose was recovered from the WL excreta and 82% from the RIR/BPR. Rapid elimination of 65% (WL) and 43% (RIR/BPR) of the dose within the first 8 hours after administration suggests similar excretion patterns for both breeds. From analysis of the excreta it was concluded that WL hens transformed 16% of the administered dose to ^{14}C-labelled metabolites, while RIR/BPR hens transformed only 3.4%. Table 6 shows the percentage of the administered dose for each of the compounds isolated from the extractable part of excreta.

Radiocarbon in the eggs was 0.79% and 0.30% of the administered dose for WL and RIR/BPR hens respectively. Peak levels were 0.25 mg/kg diflubenzuron equivalents on the third day in WL eggs and 0.16 mg/kg on the sixth day in RIR/BPR eggs. WL egg residues were consistently higher than RIR/BPR for 4 days after treatment but seemed to peak in about half the time. TLC and HPLC analysis of the ethyl acetate extracts revealed only unchanged diflubenzuron.

Total residues in the tissues are shown in Table 7. Because of the great difference in bird size, RIR/BPR being about twice as heavy as WL, the higher residues found in the RIR/BPR hens seem to account for the smaller percentage of the administered dose in their excreta. Levels of radioactivity in the tissues were too low for further characterization.

Microsomes were prepared from 25% homogenates of the livers by subcellular fractionation using an ultracentrifuge (10 min 600 x g, supernatant 30 min 18000 x g, supernatant 90 min 105000 x g). The resulting microsomal pellet was washed and resuspended in phosphate buffer. Aliquots of this preparation were incubated with [^{14}C]diflubenzuron in the presence of an NADPH-generating system for 90 min at 37°C. Diflubenzuron and metabolites were extracted with ethyl acetate. Part of the extract was radioassayed, and the remainder chromatographed by TLC with autoradiography. Radioactive spots were scraped from the plates and counted for quantification.

In vitro incubations of diflubenzuron with WL and RIR/BPR liver microsomal preparations showed in both cases about 10% conversion to metabolites. These were tentatively identified as DFBAM, CPU, DFBA and PCA. Differences between the WL and RIR/BPR livers were much lower than the differences between the egg residue levels.

Table 6. Quantification and TLC characterization of ^{14}C residues extracted from hen excreta.

Compound or fraction	% of dose	
	WL	RIR/BPR
I, diflubenzuron	50	63
II, unknown	1.2	
III, unknown (2-OH-DFB)	1.2	0.50
IV, unknown (3-OH-DFB)	1.0	0.51
V, PCA	0.44	0.58
VI, DFBAM	2.0	
VII, unknown [*N*-(4-chlorophenyl)formamide?]	0.35	0.32
VIII, CPU	3.1	0.38
IX, unknown	0.48	
X, unknown	0.48	0.35
XI, DFBA	1.4	0.22
XII, origin	4.3	0.56
lost during fractionation	0.83	0.07
total (organic phase)	67	67
aqueous phase	7.8	11
unextractable	17	4.2

Table 7. Residual ^{14}C in hen tissues, at 12 (WL) or 13 (RIR/BPR) days after administration of a single dose of 5 mg/kg bw.

Sample	WL, mg/kg diflubenzuron equivalents	RIR/BPR, mg/kg diflubenzuron equivalents
fat	0.007	0.043
liver	0.063	0.15
kidney	0.19	0.14
ovary with internal eggs	0.16	0.092
breast muscle	0.003	0.030
egg shells	0.40	nd

nd: below detection limit

Seventy-eight laying hens (breed not specified) were grouped into a control (12 hens) and three test groups (22 hens each) (Smith and Merricks, 1976b, non-GLP). [^{14}C]diflubenzuron, equally labelled in both phenyl moieties (radiochemical purity not reported), was given at dose levels equivalent to 0.05, 0.5, and 5 ppm in the feed in one capsule a day for 1 to 28 days at specific activities of 17.42, 1.76 and 0.321 mCi/mmol respectively. The authors do not state whether the doses were expressed on the dry weight. Assuming a body weight of 1.9 kg, this corresponds to dose levels of about 0.003, 0.03, and 0.3 mg/kg bw per day. Eggs were collected daily. Two hens in each group were killed on days 1, 3, 7, 10, 14, 17, 24 and 28 during the dosing period of 28 days and after a 7- or

14-day withdrawal period. Tissues (fat, leg muscle, breast muscle, liver, kidney) and eggs were stored frozen (time and temperature not reported). Samples were analysed for ^{14}C by combustion and LSC. Tissues from 4 hens in the 5 ppm group killed on day 7 were used for metabolism studies and therefore received [^{14}C]diflubenzuron at a higher specific activity (12.43 mCi/mmol). Tissues and eggs were extracted with ethyl acetate, the extract was purified by silica gel column chromatography, and in the case of eggs HPLC, after which metabolites were separated and identified by TLC (reference compounds CPU, PCA and DFBA). In eggs from the four hens dosed at 5 ppm and killed on day 7 diflubenzuron was also identified by MS.

No information was given on the excretion routes of the radiolabel. No effect of the treatment on egg production was observed. A plateau level of ^{14}C was reached in all tissues and eggs between days 1 and 10 of exposure at all dose levels. Table 8 shows the total residue levels in all groups. The authors concluded that a linear relationship between dose level and residue level existed for kidney, liver and fat, and an exponential relationship between dose level and residue level at plateau for eggs. After 7 days of withdrawal, residues in all tissues and eggs were below the limit of detection (<0.0006 mg/kg at 0.05 ppm, <0.005 mg/kg at 0.5 ppm and <0.03 mg/kg at 5.0 ppm).

Tissues and eggs from hens exposed for 7 days at 5.0 ppm were extracted with ethyl acetate. The compounds found in the extracts are shown in Table 9. The main metabolite was CPU.

Table 8. ^{14}C residues in hen tissues and eggs.

Sample	^{14}C range found between 1 and 28 days of exposure, mg/kg as diflubenzuron		
	0.05 ppm	0.5 ppm	5.0 ppm
fat	<DL-0.018[1]	<DL-0.033	0.078-1.2
kidney	<DL-0.0026	<DL-0.013	0.068-0.34
liver	<DL-0.0026	<DL-0.044	0.059-0.45
breast muscle	<DL-0.0017	<DL	<DL-0.054
leg muscle	<DL-0.0016	<DL	<DL-0.099
eggs	<DL-0.0029	<DL-0.10	<DL-0.83

DL = 0.0006 mg/kg at 0.05 ppm; 0.005 mg/kg at 0.5 ppm and 0.03 mg/kg at 5.0 ppm

[1] This sample assumed to have been contaminated at slaughter. Next highest level was 0.0067 mg/kg diflubenzuron equivalents

Table 9. Levels of [^{14}C]diflubenzuron and its metabolites in poultry tissues and eggs, after 7 days of a 5.0 ppm dose.

Sample	TRR (mg/kg diflubenzuron equivalents)	Ethyl acetate extracts, % of TRR in sample				Unextractable, % of TRR
		diflubenzuron	CPU	DFBA	unknown	
fat	0.27	100	0.0	0.0	0.0	0.0
leg muscle	0.090	66	13	6.8	1.6	12
breast muscle	0.031	63	22	9.2	5.3	0.0
liver	0.26	19	50	7.4	5.2	19
kidney	0.17	24	40	0.0	0.0	36
eggs	0.32	69	11	3.7	0.0	16

Ross Hisex laying hens were dosed by gavage for 10 consecutive days (Gifford and Dunsire, 1994, GLP). The study report as submitted was incomplete: appendices 2-8 were missing. [^{14}C]diflubenzuron, equally labelled in both phenyl moieties (radiochemical purity 97.3-99.5%), was given to 6 hens at 1 mg/kg bw/day, and to 3 hens at 8 mg/kg bw/day at specific activities of 0.72 and 0.037 MBq/mg respectively. Three control hens received the vehicle. The doses were given in two capsules/day. Excreta and eggs were collected. Two hours after the last dose the hens of all groups were killed and tissues (among others breast muscle, thigh muscle, liver, kidney, subcutaneous and abdominal fat) partially formed eggs and eggs were collected. Storage conditions were not reported. Samples were analysed for radioactivity by combustion and LSC.

Elimination of radioactivity in excreta (about 85% in the 1 mg/kg bw group and 87% in the 8 mg/kg bw group) was rapid and remained constant throughout the study. Of the total administered radioactivity 4.0 and 4.3% was recovered from tissues of the 1 and 8 mg/kg bw groups respectively; egg yolk contained 0.36 and 0.34% respectively. Radioactivity in the yolk accumulated to a plateau after the 15th dose of 0.82 mg/kg diflubenzuron equivalents in the 1 mg/kg bw group and 7.3 mg/kg in the 8 mg/kg bw group. In the edible tissues, the highest levels of radioactivity were observed in fat, liver, and partially formed eggs (Table 10).

Table 10. Radioactivity in hen tissues after repeated dosing of [^{14}C]diflubenzuron expressed as mg/kg diflubenzuron equivalents; results are the mean of 6 hens for the low-dose group and 3 hens for the high-dose group.

Sample	1 mg/kg bw/day	8 mg/kg bw/day
liver	0.60	2.9
kidney	0.44	1.9
abdominal fat	0.98	3.5
subcutaneous fat	0.91	6.2
breast muscle	0.099	0.5
thigh muscle	0.16	0.5
partially formed eggs	0.53	4.4

The target doses for this study were 1 and 10 mg/kg bw/day. As there was insufficient radioactive material, the high doses were reduced from about 10 mg/kg bw (doses 1–5) to about 9 mg/kg bw (doses 6–11) to about 8 mg/kg bw (doses 12-20). Since the levels in tissues will be mainly determined by the doses at the end of the treatment period, the high dose is taken to be 8 mg/kg bw/day.

Levels of diflubenzuron and metabolites were quantified in liver, kidney, muscle, fat, skin, and egg yolk and white by De Bie *et al.* (1996, GLP). Samples were stored homogenized at –20°C for 11-15 months, then mixed with DMSO and silica and the mixture used as an HPLC pre-column connected to one of two C-8 HPLC systems (system I 5 min 0.3% ammonium hydrogen phosphate, 0-60% methanol linear gradient in 30 min, 15 min 60% methanol, 25 min 100% methanol; system II 32 min 65% 1:10 dioxane in water/35% ACN followed by 35%-60% ACN linear gradient in 23 min); both systems with off-line LSC detection. Both gradient systems had >95% extraction efficiency for CPU, PCA, PCAA and diflubenzuron added to control samples. Compounds in the HPLC fractions were identified by co-chromatography of reference compounds (diflubenzuron, CPU, PCA, PCAA) in both gradient systems.

Most runs showed good recovery of the total radioactivity (>90% of the TRR); 2 of 36 runs showed low recoveries (43% and 62% of the TRR). The amounts of radioactivity associated with diflubenzuron and its metabolites as percentages of the total radioactivity measured in the tissues (Table 11) have been calculated by the present reviewer and are corrected for the variable extraction recoveries.

Since the unidentified metabolites in liver and kidney accounted for 30-50% of the residual radioactivity in the low dose group, these fractions were analysed by LC-MS-MS and GC-MS (after methylation with diazomethane in ether). The fractions were purified and separated into two polar fractions, identified in kidney as CPU and DFBA. Of the two polar fractions in liver, one was identified as CPU and the other remained unidentified.

Table 11. Amounts of parent compound and metabolites in tissue samples taken 2 hours after the last oral dose of diflubenzuron.

Sample	Dose level (mg/kg bw/day)	^{14}C, mg/kg diflubenzuron equivalents and (% of total radioactivity in sample)				sum of residues as % of total ^{14}C in sample	total residue, mg/kg diflubenzuron equivalents
		CPU	PCAA	PCA	diflubenzuron		
liver	1	0.12 (20)	0.015 (2.6)	0.018 (3.1)	0.20 (34)	59	0.67
	8	0.79 (22)	nd	0.048 (1.3)	1.8 (49)	72	4.0
kidney	1	0.089 (23)	nd	0.014 (3.6)	0.048 (12)	38	0.44
	8	0.50 (28)	nd	nd	0.40 (22)	50	2.0
muscle	1	0.020 (14)	nd	nd	0.10 (71)	85	0.15
	8	0.14 (15)	nd	nd	0.72 (76)	91	1.0
fat	1	0.008 (0.8)	0.005 (0.5)	nd	0.99 (98)	99	1.0
	8	0.051 (0.6)	0.026 (0.3)	nd	7.9 (99)	100	8.1
skin	1	0.016 (3.8)	nd	nd	0.38 (90)	94	0.42
	8	0.082 (2.6)	nd	nd	3.0 (94)	96	3.3
egg yolk (post-dose)	1	nd	nd	nd	0.26 (75)	75	0.81
	8	0.56 (11)	nd	nd	4.2 (80)	91	5.6
egg white (post-dose)	1	nd	0.007 (37)	nd	0.001 (5.3)	42	0.024
	8	nd	nd	nd	nd	nd	0.29

nd: not detected

Storage stability was investigated in this study. In egg yolk and liver diflubenzuron, CPU, PCAA and PCA were stable for 12 months, but in thigh muscle although diflubenzuron and CPU were stable for 12 months, PCA levels decreased to 0% and PCAA levels increased to 168% (perhaps from transformation of PCA). In muscle, the amount of PCA may therefore be underestimated.

Pigs. A Poland-China Duroc female pig of 46 kg was given by capsule a single dose of 5 mg/kg bw ^{14}C-diflubenzuron (equally labelled in both phenyl moieties, specific activity 17.42 mCi/mmol, radiochemical purity >99%), which is about 10 times the dose needed to control housefly larvae in the excreta (Opdycke, 1976; Opdycke *et al.*, 1976, 1982a, all non-GLP). Urine and faeces were collected at 12-hour intervals until slaughter after 11 days. Samples of liver, kidney, fat, internal and external muscle, skin and bone were taken for ^{14}C measurement by combustion and LSC. Samples were stored at –20°C (time not reported). Faeces were extracted with ethyl acetate and urine was extracted with diethyl ether. Metabolites were characterized by TLC co-chromatography and HPLC with reference compounds CPU, PCA, DFBA, 2-OH-DFB and 3-OH-DFB.

In the course of the 11 days after treatment, 82% of the administered radioactive dose was excreted in the faeces, and over 5% was recovered in the urine. 78% of the administered dose was recovered by day 4. The highest ^{14}C residue in edible tissues was 0.30 mg/kg in the fat (Table 12).

In faeces, all radioactive residues were identified as diflubenzuron. Identification of the metabolic products found in the urine revealed CPU, DFBA, PCA, and DFBAM, (Table 13). Although these findings indicate that most of the absorbed diflubenzuron is metabolized by pigs, the bulk of the dose eliminated in the faeces is unchanged diflubenzuron.

Table 12. Radioactivity in pig tissues, 11 days after administration of a single dose of 5 mg/kg bw.

Sample	mg/kg diflubenzuron equivalents
omental fat	0.30
subcutaneous fat	0.20
liver	0.23
kidney	0.11
longissimus dorsi muscle	0.050
latissimus dorsi muscle	0.042

Table 13. Quantification and TLC characterization of ^{14}C-diflubenzuron and metabolites in pig urine.

Compound	% of dose	% of TRR in urine[1]
I, diflubenzuron	0.45	7.5
II, unknown	0.68	11
III, unknown	1.5	25
V, PCA	1.0	17
VI, DFBAM	0.83	14
VIII, CPU	0.82	14
X, unknown	0.31	5.2
XI, DFBA	0.29	4.8
XII, origin	0.10	1.7

[1] calculated by reviewer

Five female and four castrated male pigs (Landrace) weighing between 20 and 23 kg were dosed by capsule with [^{14}C]diflubenzuron (equally labelled in both phenyl moieties, radiochemical purity >99%, specific activity 2.33 mCi/g) twice a day for 10.5 days (Cameron and Phillips, 1985, non-GLP). Each dose was 7.5 mg. As the pigs were fed 1 kg dry feed per day, this represented a daily intake of 15 mg/kg dw feed, but as they gained weight during the study (up to 31 kg), the dose decreased from 0.58-0.68 mg/kg bw/day on the first day to 0.48-0.58 mg/kg bw/day on day 10. Urine and faeces were collected every 24 hours throughout the dosing period and the 7-day withdrawal period from pigs 4, 5, 7 and 8. The pigs were slaughtered in 3 groups of 3: two females and one male on day 11, at peak plasma concentration following the final dose: one female and two males on day 18, 7 days after the last dose and two females and one male on day 25, 14 days after the last dose. Samples of tissues and organs (18) were collected and stored at -20°C (time not reported). Radioactivity was measured by LSC after combustion.

The total excretion was 88-92% of the administered doses (7 days after the last dose) of which 69-79% was in the faeces and 8.6%-10% in the urine. 1.5%-10% was recovered from cage wash and debris. The highest tissue residues were in the liver. Relatively high levels of radioactivity were present in the bile of pigs slaughtered at peak plasma levels after the final dose. By 7 days after the final dose the levels of radioactivity in all tissues except the liver were insignificant. Table 14 shows the total radioactive residue levels in the tissues: values are the means of 3 pigs, since no differences between males and females were observed. In several cases values are included that were estimated from results below the LOQ (30 dpm above the background count). These are indicated by superscripts showing the number of results used. Values derived from 3 results above the LOQ are in bold.

Table 14. Radioactivity in pig tissues after administration of 15 mg [^{14}C]diflubenzuron/day for 10.5 days.

Sample	^{14}C, mg/kg as diflubenzuron		
	6 hours after last dose (mg/kg)	7 days after last dose (mg/kg)	14 days after last dose (mg/kg)
skeletal muscle: fore	0.009[1]	<DL	<DL
hind	0.011[2]	<LOQ	<DL
fat: renal	0.017[2]	<DL	<DL
subcutaneous	0.011[2]	<DL	<DL
omental	0.009[2]	<DL	<DL
liver	**0.11**	**0.027**	<LOQ
kidney	**0.062**	<LOQ	<LOQ

LOQ: 30 dpm above background
DL: 10 dpm above background
[1] mean of 2 samples
[2] mean of 3 samples

Diflubenzuron was determined in the liver and kidneys from the 3 animals slaughtered 6 hours after the last dose by GC-ECD method LAI 3-86-18 (De Wilde and Buisman, 1986, non-GLP). The levels in the liver and kidney were below the LOQs (0.01 and 0.03 mg/kg respectively).

The levels of the parent compound and the putative metabolites DFBA, DFHA, DFBAM, CPU, PCA, 2-OH-DFB, 3-OH-DFB and *N*-acetyl-4-chlorophenylurea were determined in the liver and kidneys of the 3 animals slaughtered 6 hours after the last dose and in the excreta collected on days 10 and 11 of the 4 pigs slaughtered 7 days after the last dose (Van Berkel *et al.*, 1986). Liver and kidney were solubilised with subtilisin A (3 hours at 60°C, pH 9) and the supernatant was injected onto a Nucleosil RP-8 pre-column. Urine was injected either directly or after treatment with β-glucuronidase and arylsulfatase. Faeces was mixed with silica and the mixture used as a pre-column. The analytical system consisted of a Zorbax RP-8 column with a gradient of 100% water up to 30% w/v ammonium hydrogen phosphate in 100% methanol with off-line LSC.

In the faeces, almost all the radioactivity could be attributed to the unchanged parent compound. In urine, only 1-2% was from the parent. The main metabolites were formed by cleavage of the urea bridge. Enzymatic treatment of the urine effected a minor change in the most hydrophilic residues, resulting in the appearance of an extra peak (15% of the TRR) which did not match any of the reference compounds. In liver and kidney the metabolite pattern was qualitatively similar to that in urine (Table 15). None of the metabolites matched the retention time of PCA.

Table 15. Diflubenzuron and metabolites in extracts of liver, kidney, faeces and urine as % of TRR in the sample.

Compound	Liver	Kidney	Faeces	Urine
DFBA	30	55		55
DFHA	20	10		20
DFBAM				5
CPU				10
diflubenzuron			99	1
Unknown extractables	50[1]	35[2]	1	5[3]

[1] one peak which eluted without retention, indicating extreme hydrophilicity

[2] four peaks of which one (10%) eluted without retention.

[3] 3 peaks (1%, 2%, 2% of TRR).

The metabolic pathways in livestock are shown in Figure 1.

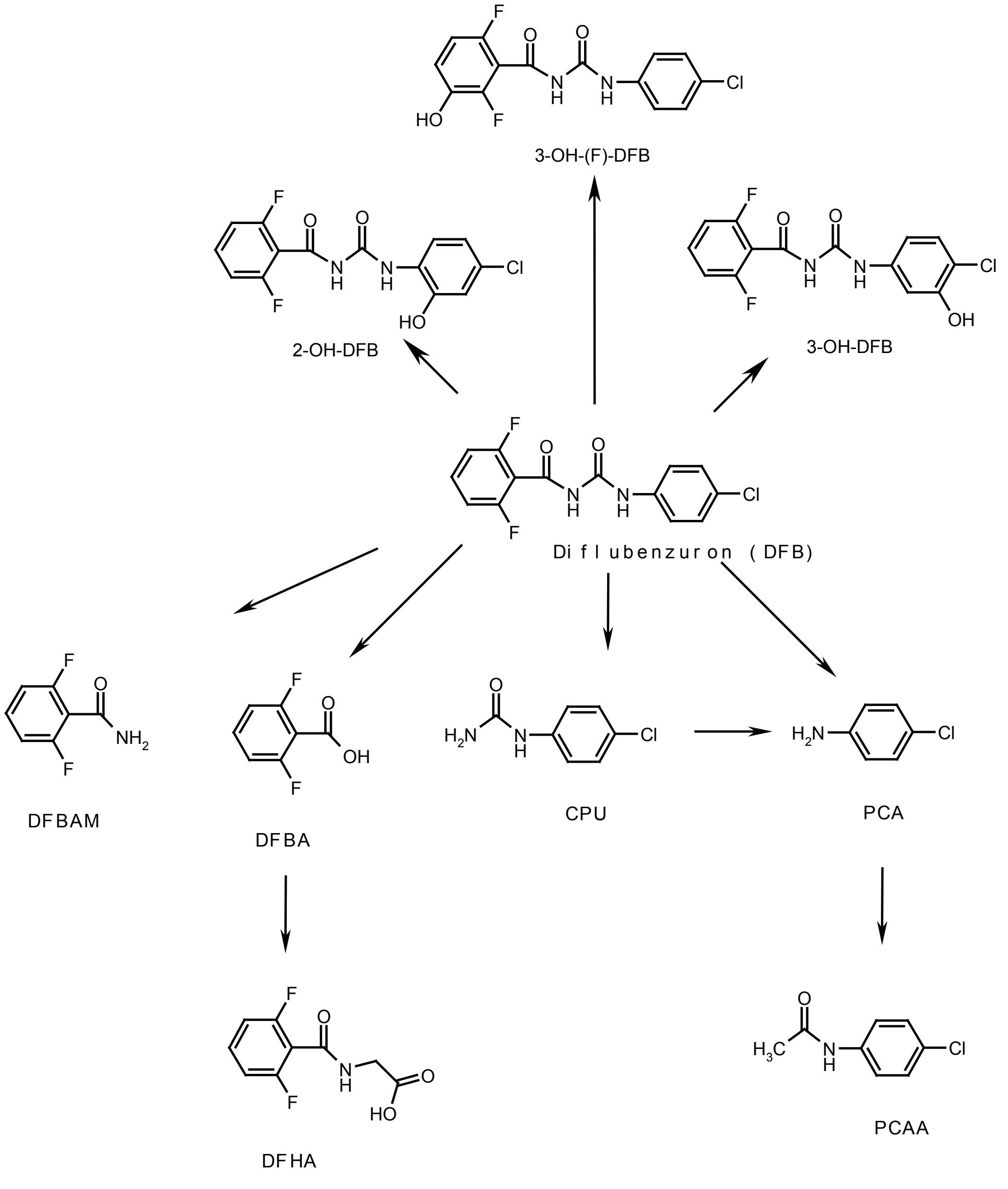


Figure 1. Metabolism of diflubenzuron in livestock.

Plant metabolism

The Meeting received information on the fate of diflubenzuron after foliar application to maize, soya bean, cabbage, apple, cotton and rice, application to apple and orange fruits and soya bean pods, soil application to cotton, surface water treatment of rice and wheat, and compost and/or casing treatment of mushrooms. Information was also received on the fate of diflubenzuron after incubation on bean leaf disks and after injections into the stem and leaves of lima beans, and on CPU and DFBA uptake by roots and stems from nutrient solutions.

Maize, soya bean, cabbage and apple (foliar spot treatment). Apple trees (Golden Delicious), maize (Caldera), soya bean (Glycine Max) and cabbage plants (Canson) were grown in a semi-open greenhouse and young seedlings of cabbage and maize (2-3 weeks old) were grown in a climate cabinet. Leaves were treated spotwise by syringe with an aqueous suspension (<2 µm particles) of diflubenzuron in which the difluorobenzoyl moiety was labelled with ^{3}H (specific activity 31 mCi/g; radiochemical purity not reported) and the chloroaniline moiety with ^{14}C (specific activity 6.3 mCi/g; radiochemical purity not reported). The dosage rate was comparable to 0.3 kg ai/ha. Leaves of seedlings were sampled 0, 1 and 2 weeks and mature leaves 0, 2, 4, 9 and 16 weeks after application. Soya bean seeds and maize grain were sampled after 16 weeks and apples (fruit) after 21 weeks. Storage conditions were not reported (Nimmo and De Wilde, 1974, non-GLP).

Homogenized leaves, beans and grain were extracted with ACN; apples were extracted successively with ACN and methanol/water (1:1). Extracts and unextractable residues were subjected to combustion and LSC. Diflubenzuron was determined radiometrically. Extracts were characterized by TLC (silica gel; 2 solvent systems) with detection by UV absorption and autoradiography. Reference compounds were diflubenzuron, CPU and PCA.

Of the total the applied radioactivity 82%-109% of both ^{3}H and ^{14}C was recovered; 95%-100% of the TRR was extractable and <0.5%-5% was bound (Table 16). The ^{3}H residues were not characterized. The extractable ^{14}C residues were identified as the parent compound (91%-109% of the TRR), except in extracts of apple leaves after 16 weeks where 84% of the TRR was diflubenzuron and 12% comprised unknown residues. The average concentration of diflubenzuron corresponded to 10, 30, 60, and 90 mg/kg in mature leaves of cabbage, maize, apple and soya bean respectively. Autoradiography of TLC plates showed trace amounts of 2 unidentified breakdown products (neither CPU nor PCA) after 2 weeks and longer. Autoradiographs of freeze-dried leaves of soya bean and maize gave an exact record of the manual spotwise application of the diflubenzuron dispersion. No radioactivity was detectable 16-21 weeks after application in maize grain, soya bean seeds or apple fruit (<0.001, <0.02 and <0.005 mg/kg respectively).

No significant degradation or translocation of diflubenzuron had taken place on the leaves of soya bean, maize or cabbage up to 16 weeks after application, but some might have occurred in apple leaves. When applied to the leaves, diflubenzuron is not translocated to maize grain, soya bean seed or apple fruits.

Table 16. Distribution of radioactive residues after treatment of leaves of soya bean, apple, cabbage and maize.

Sample	WAT[1]	total ^{14}C (% of applied)	extracted (% of TRR)	diflubenzuron (% of TRR)	bound (% of TRR)	total ^{3}H (% of applied)	extracted (% of TRR)	bound (% of TRR)
soya bean mature leaves	0	99	100	100	0.0	101	100	0.0
	2	102	100	102	0.5	104	100	0.5
	4	104	96	91	3.8	106	96	3.8
	9	97	96	99	4.1	98	97	3.1
	16	-	-	-	-	-	-	-
apple mature leaves	0	101	100	100	0.0	102	100	0.0
	2	99	96	94	4.0	101	96	4.0
	4	91	97	93	3.3	95	97	3.2
	9	88	98	101	2.3	90	98	2.2
	16	82	96	84	3.7	90	97	3.3
maize mature leaves	0	101	100	100	0.0	105	100	0.0
	2	103	99	104	1.0	105	99	1.0
	4	98	97	100	3.1	101	97	3.0
	9	99	95	96	5.1	100	95	5.0
	16	86	98	105	2.3	90	98	2.2
cabbage mature leaves	0	101	100	100	0.0	101	100	0.0
	2	95	96	99	4.2	100	96	4.0
	4	100	97	96	3.0	105	97	2.9

Sample	WAT[1]	total ^{14}C (% of applied)	extracted (% of TRR)	diflubenzuron (% of TRR)	bound (% of TRR)	total ^{3}H (% of applied)	extracted (% of TRR)	bound (% of TRR)
	9	105	95	95	4.8	109	95	4.6
	16	-	-	-	-	-	-	-
maize seedlings	0	99	100	100	0.0	101	100	0.0
	1	95	100	100	0.0	na	na	na
	2	101	100	109	na	101	100	na
cabbage seedlings	0	100	100	100	0.0	101	100	0.0
	1	99	95	101	5.1	99	96	4.0
	2	96	97	105	3.1	101	970	3.0

[1] weeks after treatment

- : treated leaves decayed

na: not analysed owing to technical difficulties

Apple (spotwise foliar and fruit treatment). Apple trees (Golden Delicious) were grown in a semi-open greenhouse. Leaves and fruits were treated spotwise by a syringe with a dispersion (<10 µm) of diflubenzuron, in which both the phenyl rings were labelled with ^{14}C (specific activity 5.6 mCi/g; radiochemical purity 99.1%). The dosage rate was not reported; the dispersion was prepared in a mixture of dispersing agent (Polyfon H), wetting agent (Sellogen HR) and detergent (Citowet) to obtain sufficient wetting of the leaves. Treated leaves were sampled 0, 4, 8 and 14 weeks after application. Untreated new-grown leaves were sampled 8 and 14 weeks, and fruits 14 weeks after application. Storage conditions were not reported (Nimmo *et al.*, 1978, non-GLP).

Apples were washed with ACN/methanol (1:1). Homogenized washed apples and homogenized leaves were extracted with ACN followed by reflux for 30 min with methanol/water (1:1). Rinsing solvent, combined extracts and unextractable residues were subjected to combustion and LSC. Diflubenzuron and CPU were determined by reversed isotope dilution analysis. Extracts were characterized by TLC as above. Reference compounds were diflubenzuron, CPU, PCA, DFBA, 2-OH-DFB and 3-OH-DFB.

Of the total the applied radioactivity 90%-111% was recovered. In leaves 99% of the TRR was extractable, and <1% of the TRR was bound; in fruits 96% was extractable and 4.2% bound; 91% of the TRR in the fruits was recovered by rinsing with ACN and methanol. The extractable ^{14}C residue in leaves and fruits consisted of the parent compound (96%-99% of the TRR); CPU, DFBA, PCA, 2-OH-DFB and 3-OH-DFB were not found. Samples of newly grown leaves appeared to contain <0.5% of the applied radioactivity.

The applied diflubenzuron remains almost completely at the site of application, and thus does not penetrate the fruit.

Apple (spotwise foliar and fruit treatment). In a greenhouse, 28 fruits of 5 apple trees (Rainha) and four leaves surrounding them were treated spotwise by syringe with diflubenzuron in which both the phenyl rings were labelled with ^{14}C (WP-formulation, specific activity 19.5 mCi/g; radiochemical purity 98.4%). The dosage rate was not reported. Fruits and leaves were harvested 9 weeks after the application and analysed on the day of harvest. (Van Kampen and Joustra, 1991; Serra and Joustra, 1990, both GLP).

Four subsamples of three apples each were rinsed with dichloromethane; leaves were rinsed with ACN and apples and leaves were subsequently homogenized. Leaves were not extracted. The apple pulp was extracted twice with ACN. All extracts and the rinsing fluid were combined. The combined extracts were concentrated and after clean-up by ether extraction analysed by HPLC (RP-8 column) with LSC and UV detection. Reference compounds were diflubenzuron, DFBA, DFBAM, CPU, PCA and PCAA. Unextractable residues were subjected to combustion and LSC.

Recoveries from control fruits spiked before homogenization with 0.1 mg/kg radiolabelled diflubenzuron or 0.01 mg/kg radiolabelled CPU, PCA or DFBA were 96% for diflubenzuron, 119% for CPU, 103% for DFBA and 33% for PCA. Results were corrected for these overall recoveries (including homogenization, extraction and analysis).

Of the radioactivity applied to the whole fruits, 94%-100% (mean 97%) was recovered at harvest. The apples (whole fruit) contained on average a total of 2.1 mg/kg diflubenzuron equivalents of which 97% was the parent, while CPU, DFBA, PCA, PCAA and DFBAM were not found (each <1 mg/kg diflubenzuron equivalents) and 0.2% of the TRR was bound. Leaves were not analysed.

Treatment of fruits and leaves did not lead to the formation of detectable amounts of metabolites in whole apple fruit.

<u>Orange (spotwise foliar and fruit treatment)</u>. In a greenhouse, 260 oranges of 16 orange trees (Hamlin round) and four leaves surrounding them were treated twice with an acetone solution of diflubenzuron in which both the phenyl rings were labelled with ^{14}C (specific activity 19.5 mCi/g; radiochemical purity 98.4%). The dosage rate was comparable to 2 applications at 0.35 kg ai/ha with an interval of 14 days. The solution was applied as streaks with a syringe. Fruit and leaves were harvested 21 days after the second application and were stored at –20°C for 2-6 days (Nigg, 1989; Joustra *et al.*, 1989). GLP was claimed for the analytical part of the study, not for the field part.

Three subsamples of three oranges each were rinsed with dichloromethane; leaves were rinsed with ACN and oranges and leaves were subsequently homogenized. Leaves were not extracted. The pH of the orange pulp was adjusted to 8 (with 5 M KOH) and the pulp was extracted three times with dichloromethane-ACN (3:2). The pH of the pulp was then adjusted to 2 with 5 M HCl and the pulp extracted twice with dichloromethane-ACN (3:2). All extracts and the rinsing fluid were combined. The combined extract was concentrated and extracted three times with n-hexane to remove the citrus oil. The remaining dichloromethane-ACN layer was analysed. Extracts and unextractable residues were subjected to combustion and LSC. Extracts were characterized by HPLC (Nucleosil RP-8 column) with LSC and UV detection. Reference compounds were diflubenzuron, CPU, DFBA and PCA.

Recoveries from control fruits spiked before storage with 0.01 mg/kg radiolabelled diflubenzuron, CPU, PCA or DFBA were 78% for diflubenzuron, 65% for CPU, 17% for DFBA and 9% for PCA as determined by HPLC. Results were corrected for these recoveries.

Of the radioactivity applied to the whole fruits 86%-99% (mean 93%) was recovered. The oranges (whole fruit) contained on average a total of 0.66 mg/kg diflubenzuron equivalents of which 96% was the parent, while CPU, DFBA and PCA were not found (each <1 mg/kg diflubenzuron equivalents) and 5.4% of the TRR was bound. Leaves were not analysed.

Treatment of the fruit and leaves did not lead to the formation of detectable amounts of metabolites in whole oranges.

<u>Soya bean (spotwise foliar and pod treatment)</u>. In a semi-open greenhouse, leaves and/or developing pods of soya bean plants (Glycine Max, var. Evasoy) were treated twice with diflubenzuron (WP formulation), in which both phenyl rings were labelled with ^{14}C (specific activity 19.6 mCi/g; radiochemical purity 99.5%). The dosage was comparable to 1-2 x 0.07 kg ai/ha with an interval of 3 weeks. At the first application only the leaves were treated. At the second application (day 18) 50% of the leaves were treated again; the other leaves and pods were treated for the first time. The radiolabelled suspension was applied spotwise with a syringe. Leaves were sampled on days 1, 18 and 62 (at harvest); pods were sampled on days 18 and 62. Mature pods were separated into seeds and hulls. The untreated plant parts (vines and pods) were analysed as well. Samples were extracted within 24 hours after sampling (Thus and Van der Laan-Straathof, 1993a, GLP).

Homogenized samples were extracted successively with ACN (twice) and methanol and the extracts pooled. Extracts and unextractable residues were subjected to combustion and LSC. Extracts were characterized by HPLC (C-18 column) with LSC and UV detection. Reference compounds were diflubenzuron, CPU, DFBA and PCA.

Of the total the applied radioactivity, 89%-105% was recovered, of which >99% was found in the treated leaves and pods and less than 0.2% in the untreated vines and pods. >99% of the radioactivity recovered from the treated pods was found in the hulls and less than 0.2% in the seeds. Autoradiographs of freeze-dried leaves of soya bean (day 62) gave an exact record of the manual spotwise application of the diflubenzuron dispersion.

In the leaves 94%-104% of the TRR was identified as the parent compound; the remaining residue consisted of bound residues (1.2%-2.2% of the TRR). At days 1 and 18 no significant amounts of metabolites could be detected (<0.2% of the TRR); at harvest (day 62) trace amounts of at least 5 metabolites were detected, none of which corresponded to DFBA, CPU or PCA (Table 17).

In the immature pods (day 18) and mature hulls (day 62) 90%-103% of the TRR was identified as the parent compound; the remainder was bound (0.5%-1.3% of the TRR). On day 18 metabolites were not detected (<0.2% of the TRR), but on day 62 trace amounts of at least 2 metabolites were detected, none of which was DFBA, CPU or PCA.

It was concluded that diflubenzuron is not translocated nor significantly metabolized in soya bean plants.

Table 17. ^{14}C residues in soya bean leaves, pods and hulls, expressed as % of recovered ^{14}C.

Sample	day	DAT	Total ^{14}C µg as diflubenzuron	% diflubenzuron	% unknown	% bound
leaves	1		480.0	94%	<0.2%	2.1%
	18	0	981.5	104%	<0.2%	1.2%
	62	44	915.7	100%	<0.2%	2.2%
pods	18	0	60.3	103%	<0.2%	0.5%
hulls	62	44	52.7	90%	<1%	1.3%

DAT: days after last treatment: leaves were treated twice; pods and hulls were treated once

Bean leaf discs. ^{14}C-labelled diflubenzuron (label position, specific activity and radiochemical purity not reported) was applied to the under epidermis of dwarf bean leaf discs 16 mm diameter in agar cylinders 6 mm diameter at a concentration of 0.08 g/l diflubenzuron in the presence of 1% Tween 20 to prevent crystallisation and 0.008% DMSO (solvent for diflubenzuron). The experiment lasted 24 hours, 16 hours under illumination (intensity ca 10.000 lux) and 8 hours in the dark. The leaf discs were placed on damp filter paper in a petri dish. The agar cylinders were removed after 24 hours and portions of the epidermis were stripped from the leaf discs to differentiate between radioactivity in and on the leaf. Leaf discs were fixed, freeze-dried and examined by autoradiography (Den Hartog and Nimmo, 1975, non-GLP).

No blackening of the X-ray film was observed outside the spots where the agar cylinders had been, nor where the epidermis and cuticule were removed, indicating that diflubenzuron did not penetrate the leaf discs.

Citrus (foliar spray). Six miniature citrus plants (Carrizo Citrange) were sprayed in a greenhouse with diflubenzuron in which both the phenyl rings were labelled with ^{14}C (specific activity 19.6 mCi/g; radiochemical purity 99.5%), at a dosage rate of 0.35 kg ai/ha (WP-formulation; 560 l/ha). Two simulated 1-cm rain showers (tap water pH 6.9) were applied 1, 2, 4, 6, 8 and 24 hours after treatment. The wash-off from each plant canopy was collected and the ^{14}C quantified by LSC. The ^{14}C in 5 leaves from each plant collected before and after treatment was also quantified by combustion and LSC. Samples were stored at 8ºC (time not reported) (Nag, 1997, non-GLP).

56%-103% of the applied radioactivity was recovered (Table 18). The first 1-cm washes "rain showers" removed 40%-65% of the TRR and the second 16%-36%, giving combined totals for 2 cm of wash water of 57%-87% of the TRR. When treated citrus leaves were soaked in tap water for 24 hours, removal of radioactivity was essentially quantitative (96% of the TRR). Apparently ^{14}C-diflubenzuron did not penetrate the leaves of the citrus plants.

Table 18. Wash-off of diflubenzuron from citrus leaves.

Hours after treatment	Total ^{14}C recovered (% of applied)	% of recovered ^{14}C			
		wash-off 1st cm	wash-off 2nd cm	total wash-off (2 cm)	Remaining on plant
1	94	65	22	87	13
2	91	40	34	74	26
4	103	44	36	80	20
6	94	44	27	71	29
8	56	44	22	66	34
24	86	41	16	57	43

Cotton (leaf or soil treatment). In a greenhouse, leaves of cotton plants (Stoneville 213) and bare soil (loamy sand, pH = 7.15; 2.1% om, 10.9% silt) were treated with an aqueous suspension (WP-formulation) or an oil dispersion (2% Savol) of diflubenzuron labelled in both phenyl rings (specific activity 17.4 mCi/mmol; radiochemical purity 99%). Plants were treated either once or three times at 7 day intervals; the dose rate was not reported. Treated plants were grown in vermiculite and nutrient solution. Plants treated once were sampled immediately after treatment and after 7, 14 and 28 days. Plants treated three times were sampled after each treatment (days 0, 7 and 14) and 28 and 48 days after the first treatment. For the soil experiment untreated plants 41 days old were transplanted into freshly-treated soil and harvested after 21, 42 and 89 days. At harvest, plants were divided into leaves, stems, roots and bolls plus squares. Storage conditions were not reported (Mansager *et al.*, 1979, non-GLP).

Plant samples and soil were subjected to combustion and LSC. Plant samples from foliar treatments were extracted with ACN and hot 80% ethanol, and from soil treatments with ACN alone. Soil was extracted with 80% ethanol and extracts were partitioned between dichloromethane and water. Residues in the plant and soil extracts were characterized by HPLC (C-18 and Carbowax 20-M), ion exchange chromatography (XAD-4) and TLC (silica gel; 1 solvent system). Isolated compounds were identified by MS with direct solid sample insertion. The reference compounds used were not reported.

For the foliar treatment >99% of the total the applied radioactivity was recovered from the treated leaves, 0.02%-0.05% from the stems, <0.01%-0.08% from the roots, 0.18%-0.37% from the bolls and squares, and <0.01%-0.07% from tissues developed above the treated area. Total residues recovered from stems, roots and newly developed tissues were below 0.09, 0.60, and 0.12 mg/kg dw diflubenzuron equivalents respectively. The total residues recovered from treated leaves and bolls plus squares are shown in Table 19. The radioactivity in the bolls and squares is surprising as these tissues were not exposed to radioactivity. When bolls and squares were subdivided into burrs, seed and cotton fibre, the radioactivity was mainly in the burrs.

No radioactivity was found in extracts of cotton seed (48 day harvest; TRR 0.05 mg/kg, diflubenzuron equivalents); 97% of the TRR was recovered from the solids. The ^{14}C in all the leaf and stem extracts co-chromatographed with diflubenzuron. The authors concluded that cotton plants do not absorb or translocate diflubenzuron. Residues resulting from the oil dispersion tended to be lower than those from the aqueous suspension.

Biometer flasks were used to measure the degradation of diflubenzuron in soil treated with an aqueous suspension of ^{14}C-diflubenzuron (100 g soil plus 20 ml water; in the dark, at room temperature). After 91 days, 28% of the applied radioactivity was degraded to $^{14}CO_2$ and 69% remained in the soil.

21, 42 and 89 days after soil treatment, 77%, 55% and 51% of the applied radioactivity was in the soil, 1.2%, 1.6% and 3.5% was in the plants and 21%, 43% and 45% was missing (probably mainly as $^{14}CO_2$). Of the total recovered radioactivity in the plants at these times 60%, 80% and 67% respectively was in the leaves, 32%, 12% and 24% in the roots, 7.3%, 6.8% and 4.5% in the stems and <LOQ, 0.62% and 2.8% in the bolls plus squares. The total residues recovered from leaves, stems, roots, and bolls and squares are shown in Table 20. Control plants grown in untreated soil, but in the same atmosphere containing ^{14}C-volatiles, showed the highest ^{14}C residues at 42 days after soil treatment: 0.04-0.05 mg/kg dw diflubenzuron equivalents in all tissues. As the roots in treated plants contained a lower percentage of radioactivity than the leaves, the authors concluded that [^{14}C]diflubenzuron and/or its labelled degradation products entered the roots and were translocated acropetally.

In the soil 0, 21, 42 and 89 days after treatment, 93%, 71%, 28% and 29% of the total recovered radioactivity was ethanol-extractable. The ^{14}C residue in the 0-day extract was identified as the parent compound. The ^{14}C residues in the 21-day extract were characterized as the parent (34% of the TRR), CPU (24%), and water-soluble (14%), and in the 42- and 89-day extracts as the parent (13% of the TRR), CPU (10%), DFBA (3-4%), and water-soluble (2%-3%). Of the ^{14}C residue in the 42-day leaf samples, 21% was ACN-extractable and was identified as CPU. Unextractable residues in leaves increased from 75% at 42 days to 84-93% at 89 days. The ACN extractable residues in the 89-day roots were characterized as parent (major) and DFBA (minor). The authors concluded that little if any diflubenzuron was translocated to the leaves but CPU was absorbed by the roots and translocated.

Table 19. Total ^{14}C residues in cotton after foliar treatment.

Sample	Day	^{14}C, mg/kg diflubenzuron equivalents		
		Triple treatment; aqueous suspension	Single treatment; aqueous suspension	Single treatment; oil dispersion
Leaves and stems	0 (1st treatment)	79	91	40
Leaves	7 (2nd treatment)	117	65	19
	14 (3rd treatment)	82	44	27
	28	46	41	12
	48	56	na	na
Bolls and squares	7	14	na	na
	14	2.9	na	na
	28	0.92	na	na
	48	0.09	na	na

Table 20. Total recovered residues (TRR expressed as mg/kg dw diflubenzuron equivalents) in cotton plants after soil treatment (aqueous suspension).

Sample	Day 21	Day 42	Day 89
Leaves	2.5	2.7	2.3
Stems	0.96	5.7	0.55
Roots	3.4	1.2	2.4
Bolls and squares	na	0.23	na
Burrs	na	na	0.10
Seed	na	na	0.06
Cotton fibre	na	na	0.11

Cotton, (leaf treatment and foliar spray in the field). Diflubenzuron (^{14}C-labelled in both phenyl rings; specific activity 17.4 mCi/mmol; radiochemical purity 99.0%) was sprayed onto field-grown cotton (Stoneville 213) 6 or 10 times at 5-day intervals (WP formulation, 0.07 kg ai/ha in 93.5 l/ha water and 9.35 l/ha emulsified crop oil for each application; sandy loam soil). Leaves from the 6- and 10-treatment trials were harvested 36 and 19 days after the last treatment respectively; new-grown leaves, seeds and stalks plus roots were harvested 61 and 44 days after the last treatment. Storage conditions were not reported (Bull and Ivie, 1978, non-GLP).

In another experiment, separate leaves were treated in the field (using a micropipette) with an aqueous suspension with and without 25% crop oil.

Leaves were washed with methanol, homogenized and extracted with acetone/water (9 + 1) and acetone. All cotton samples from field studies, except seeds and lint, were dried for 48 h at 40-50°C. Plant samples, solvent extracts and remaining solids were subjected to combustion and LSC. Leaf extracts were analysed by 2D-TLC with diflubenzuron and the ten potential metabolites CPU, DFBA, DFBAM, PCA, PCAA, 2-OH-DFB, 3-OH-DFB, 4-chlorophenol, 4-chloro-*N*-methylaniline, and 4-chloro-*N,N*-dimethylaniline as reference markers.

Diflubenzuron was poorly absorbed by the separately-treated cotton leaves. After 14 days (no crop oil suspension), 87% of the applied radioactivity was recovered as the parent in the external rinse, 4.8% as the parent in the internal leaf extracts, 0.5% was unextractable and 7.3% was lost. After 21 days and after a 7.6 cm rainfall (no crop oil), 23% of the applied diflubenzuron still remained on the leaf surfaces, 6.8% was recovered as parent in the internal leaf extracts, 0.4% was unextractable and 70% was lost. After 28 days exposure to summer sunlight (protection against rainfall; crop oil suspension), 56% of the applied radioactivity was recovered as the parent in the external rinse, 6.8% was recovered from the leaves and 38% was lost. No degradation products were observed in the extracts of cotton leaves. According to the authors, the losses were the result of volatilisation of diflubenzuron or other weathering factors.

Radioactive residues were low in mature seeds of cotton 61 days after 6 treatments and 44 days after 10 treatments with [^{14}C]diflubenzuron (<0.01 and 0.02 mg/kg diflubenzuron equivalents respectively), but were high in treated leaves (38 and 107 mg/kg dw diflubenzuron equivalents) at 36 and 19 DAT. Residues at 61 and 44 DAT were moderate in new-grown leaves after defoliation (0.60 and 1.4 mg/kg diflubenzuron equivalents) and in stalks, roots and calyxes (0.76 and 3.3 mg/kg diflubenzuron equivalents). The residues were all identified as the parent; no degradation products were observed.

Soya beans (foliar treatment in the field and in a greenhouse). Greenhouse- and field-grown soya bean plants (in Greenville, MS, USA) were treated at mid- to full-bloom with diflubenzuron (WP-formulation; ^{14}C-labelled in both phenyl rings; specific activity 17.8 mCi/mg; radiochemical purity 99%) at a dose rate equivalent to 1 or 2 applications of 0.06 kg ai/ha (0.01 kg ai/hl) at a 2-week interval. Samples were taken 0, 1, 2, 4, 8, 10 and 12 weeks after the first treatment. Aerial parts were separated into top and bottom halves and each half was subdivided into foliage, pods, hulls and beans (Gustafson and Wargo, 1976, non-GLP).

Samples were stored at –10°C (storage time not reported), then subjected to combustion and LSC and extracted with ACN or methanol. Extracts were counted by LSC and analysed by GC-ECD; diflubenzuron was hydrolysed to PCA and derivatized with HFBA (Thompson-Hayward Method 222). Residues in extracts were characterized by TLC (silica gel; 2 solvent systems) with diflubenzuron as the only reference marker.

In plants treated twice in a greenhouse, 0.65-3.4 mg/kg diflubenzuron equivalents was found in the foliage after 0-8 weeks. At maturity after 12 weeks residues were found in trash (4.2 mg/kg diflubenzuron equivalents), leaves (7.6 mg/kg), pods (0.36 mg/kg) and hulls (0.78 mg/kg), but not in seeds (<0.05 mg/kg).

In the plants treated in the field, the top halves contained higher residues than the bottom halves. In the top halves, twice-treated plants contained 0.63-5.0 mg/kg diflubenzuron equivalents in the foliage, 0.20-0.76 mg/kg in the hulls and no residues in immature pods and mature seeds (<0.05 mg/kg diflubenzuron equivalents). In the once-treated plants the tops contained 0.80-1.7 mg/kg diflubenzuron equivalents in the foliage, 0.07-0.15 mg/kg in the hulls and 0.06-0.15 mg/kg in the pods; no residues (<0.05 mg/kg diflubenzuron equivalents) were found in the seeds.

It was found that 57%-100% of the TRR was extractable from both greenhouse and field samples and was unchanged diflubenzuron: TLC analysis showed that the only radioactive compound in the extracts co-chromatographed with diflubenzuron, and GC-MS analysis confirmed that the compound determined by GC-ECD was the HFB derivative of PCA (parent peak at m/z 323.5).

Lima bean (stem and leaf injections). Seven-day old lima bean plants (Henderson Bush) were treated with a [^{14}C]diflubenzuron solution in acetone (labelled in both phenyl rings; specific activity 16.1 mCi/mmol; radiochemical purity 99%). The solution was applied to the upper surfaces of leaves and injected into the stems. The dose rate was not reported. After 7 days in a glasshouse, the plants were attached to glass plates and autoradiographed. In a second experiment the solution was injected into the stems and the ^{14}C distribution was determined in the leaves, stems and roots after 6 and 12 days. The storage time before analysis was not reported (Franklin and Knowles, 1981, non-GLP).

Samples were subjected to combustion and LSC, then extracted successively with ACN and water. The ACN extract was evaporated until only water remained, and this was combined with the aqueous residue from the ACN extract. The combined aqueous extracts were partitioned with ethyl acetate and both phases were analysed by LSC. The ethyl acetate extracts were evaporated to dryness and the residue dissolved in acetone. The solution was cleaned up by precipitation overnight at 5°C with an aqueous solution of 1 g/l ammonium chloride and 2% phosphoric acid. The resulting filtrate was adjusted to pH 6.5 and extracted with ethyl acetate. After drying with sodium sulfate and concentration, the extracts were characterized by 2D-TLC (silica gel) with UV and autoradiographic detection, and LSC of scraped-off spots.

When [^{14}C]diflubenzuron was applied topically to the distal tip, the margin, or the apical or lateral half of bean leaves *in situ*, there was little diffusion of radioactivity from the treated area, but movement of radioactivity was detectable after application to the basal tip and to the basal half of the upper surfaces of the leaves. When diflubenzuron was injected into the stem, there was appreciable acropetal movement of radioactive material. Movement in both leaves and stems was confined mainly to the vascular tissue.

LSC of plants after stem injection revealed that 84% of the applied radioactivity remained in the stems, the leaves contained 9% and the roots 0.2%, with 6.8% missing. In stems 6 and 12 days after exposure, 96% and 97% of the TRR respectively was organosoluble and consisted largely of the parent compound (89% and 88% of the TRR respectively). In leaves 6 days after exposure the majority was water-soluble (68% of the TRR) and the remainder was organosoluble (17%) or not extractable (15%). The organosoluble fraction consisted of the parent (1.3% of the TRR), 2-OH-DFB (2.4% of the TRR), 3-OH-DFB (8.9% of the TRR), DFBAM (0.8%), CPU (0.7%), DFBA (0.6%) and 2 unknowns (1.7% and 0.2% of the TRR). PCA was not detected.

Rice and wheat (surface water treatment). In a semi-open greenhouse, seeds of rice (Maravelli) and wheat (Orca) were cultivated in flooded buckets with sandy loam soil (pH 6.8; 2.4% om; 14% clay (<2 µm)) or sand (pH 5.3; 2.5% om; 3% clay (< 2 µm)). Young plants were treated with DFB in which the difluorobenzoyl moiety was labelled with ^{3}H (specific activity 22.9 mCi/mmol; radiochemical purity 99.0%) and the chloroaniline moiety with ^{14}C (specific activity 2.35 mCi/mmol; radiochemical purity 99.4%). The diflubenzuron was added to the irrigation water at a dose rate of 0.05 kg ai/ha. In the sandy loam experiment, samples of soil and leaves were collected 2, 5 and 10 weeks after treatment and roots after 10 weeks. In the sand experiment, soil samples were collected 6 and 18 weeks after treatment; leaves after 8 weeks and grain after 15 weeks. Storage conditions were not reported (Nimmo and De Wilde, 1976a, non-GLP).

Homogenized leaf, root, grain and soil samples were extracted sequentially with ACN and methanol/water (1:1). Extracts and unextractable residues were subjected to (combustion) LSC. DFB and CPU were determined by reversed isotope dilution analysis. Extracts were characterized by TLC (silica gel; 2 solvent systems) followed by autoradiography. Reference markers were DFB and CPU.

The recovery of the applied radioactivity from rice 10-18 weeks after the application was 99%-104% for ^{14}C and 13%-36% for ^{3}H. According to the authors, tritium losses were the result of the evaporation of water. Of the total recovered radioactivity 88%-94% (both ^{3}H and ^{14}C) was found in the soil, 1.3% of the ^{14}C and 10% of the ^{3}H in the roots and 6.2%-10% of the ^{14}C and 1.8%-6.3% of the ^{3}H in the shoots. The ^{3}H residues were not characterized.

In the soil, the total ^{14}C residues amounted to 0.028-0.092 mg/kg diflubenzuron equivalents (Table 21); 1.4%-25% of the TRR was identified as the parent compound and the remainder consisted of CPU (0.0%-53%), unknowns (0.0%-51%) and bound residues (24%-72%).

In rice and wheat leaves, the total ^{14}C residues amounted to 0.095-0.52 mg/kg diflubenzuron equivalents (Table 21). In the rice leaves 0%-16% of the TRR was identified as the parent compound; the remainder was CPU (0.0%-72% of the TRR), unknowns (16%-92%) and bound residues (1.9%-32%). In the wheat leaves, the parent compound was not detected; the residue consisted of CPU (39% of the TRR), unknowns (14%) and bound residues (46%). Rice plants did not develop panicles. The total ^{14}C residues in wheat grain corresponded to 0.018 mg/kg of which 35% was unidentified and 65% was bound.

Table 21. Mean distribution of ^{14}C and ^{3}H residues in soil, rice and wheat.

Exp.	Soil & crop	Sample	WAT	Total ^{14}C mg/kg diflubenzuron eq	% of total ^{14}C				% of total ^{3}H		
					diflubenzuron	CPU	unknown	bound	Total ^{3}H mg/kg eq	extracted	bound
I[1]	sandy loam	soil	2	0.038	25	nd	51	24	0.024	54	46
		soil	5	0.050	5.1	nd	48	47	0.020	40	60
		soil	10	0.040	9.9	27	34	29	0.013	51	49
II[2]	sand	soil	6	0.092	5.4	53	2.2	39	0.016	50	50
		soil	18	0.028	3.6	43	nd	54	0.004	50	50
III[3]	sand	soil	6	0.078	2.6	38	7.7	51	0.014	43	57
		soil	18	0.069	1.4	29	nd	72	0.012	25	75
I[1]	rice	leaves	2	0.095	16	nd	66	18	0.065	84	16
		leaves	5	0.22	6.6	nd	92	1.9	0.080	87	nd
		leaves	10	0.28	4.0	72	16	7.1	0.037	62	nd
II[2]	rice	leaves	8	0.50	nd	8.0	58	32	0.035	58	42
		leaves	15	0.26	nd	42	38	15	0.020	50	nd
III[3]	wheat	leaves	8	0.52	nd	39	14	46	0.13	77	23
		grain	15	0.018	nd	nd	35	65	0.004	72	28

[1] rice grown on sandy loam
[2] rice grown on sand
[3] wheat grown on sand
WAT: weeks after treatment
nd: not detected (<0.01 mg/kg diflubenzuron equivalents)
unknown: unidentified extractable residues calculated from total extracted by difference

<u>Rice (foliar treatment)</u>. In a greenhouse, rice seedlings (Mars) were transplanted at the 3-5 leaf stage into a loam soil (pH 7.2; 2.2% om; 25.6% clay; CEC 10.33 mequivalents/100 g) in flooded pots. Ten days later, the rice transplants received a single foliar spray (SL formulation) of [^{14}C]diflubenzuron (1:1 mixture of aniline and benzoyl ring labels; specific activity 19.1 and 20.3 mCi/g respectively;

radiochemical purity 97.7% and 97.9% respectively) at either 0.28 kg ai/ha (GAP) or 1.7 kg ai/ha. The water in the pots was not covered and the standing water was allowed to soak into the soil. Aerial parts of the plants were sampled 0, 30 and 109 days after treatment. Mature plants were separated into grain and straw and stored frozen for 24 days (temperature not reported). Homogenized grain was successively extracted twice with acidified methanol/water (80:20; 1% acetic acid) and once with acidified ACN/water (50:50; 1 M HCl), and homogenized straw twice with ACN, twice with acidified ACN/water (50:50; 1% acetic acid), and once with acidified ACN/water (50:50; 1 M HCl) (Walsh, 1997, GLP).

The remaining solids from straw were subjected to acid and base hydrolysis (1 M HCl followed by 1 M NaOH). Separate portions of the remaining solids from grain were hydrolysed with 1 M HCl, 6 M HCl, protease, 1 M NaOH or 72% H_2SO_4 (reflux). Another portion of the solids from grain was treated successively to isolate protein and starch. Proteins were isolated by heating with 5% sodium dodecyl sulfate (SDS) and 25 mM dithiothreitol at 50ºC for 16 hours; starch by refluxing for 4 hours with 1 N H_2SO_4. The remaining solids were regarded as lignin-related.

Grain, straw, extracts and unextractable residues were subjected to combustion and LSC. Extracts were characterized by HPLC (8 different column/gradient systems) with radioactive flow detection (RAD) or LSC counting of isolated fractions or by TLC (silica gel; 2 solvent systems) with autoradiographic and UV detection. Isolated metabolites were identified by MS (electron impact or chemical ionisation). Reference standards were diflubenzuron, CPU, DFBA, PCA and DFBAM. Unresolved radioactive zones derived from the extracts of grain and straw were subjected to acid and/or base hydrolysis (1 M HCl and 1 M NaOH) and analysed again by HPLC-LSC. The SDS-digest (proteins) was fractionated by GPC (Sephadex G-100). The sulfuric acid digest (starch/glucose) was acetylated and analysed for acetylated glucose by HPLC-LSC. Glucose was confirmed by phenol-sulfuric acid assay for total carbohydrate and phenyl glucozanone derivatization followed by identification based on melting point and MS.

Recoveries from control grain samples, spiked immediately before extraction with diflubenzuron (0.1 mg/kg) or PCA (0.01 mg/kg) were 94% and 98% respectively; no radioactivity was found in the acidified ACN/water extracts. Recoveries from acid hydrolysates of solid residues spiked with radiolabelled PCA (0.01 mg/kg) immediately before ethyl acetate partitioning were 95%.

The total recovered residues expressed as diflubenzuron equivalents are shown in Table 22. According to the author, the results indicate that only a very small amount of the applied radioactivity moved from the foliage to the grain and that the lower residues in the straw than in the immature plants were due to growth dilution and loss of test material from exposed leaves by senescence.

Of the total radioactivity in the grain, 26%-32% was extractable (Table 23). CPU was identified as the major metabolite (17%-22% of the TRR); the parent was present in low amounts (0.2%-0.3%). Minor metabolites were CPU conjugates (0.9% of the TRR), DFBA conjugates (3.0%), PCA (0.3%) and unknown compounds (5.0%-9.4%). The unextractable residues (60%-68% of the TRR) were characterized as ^{14}C fragments incorporated into glucose units of starch (30% of the TRR), into protein (12%) or as bound or lignin-related residues (24%). Hydrolytic treatments released 5.0%-35% of the TRR from the unextractable residues: no residues of diflubenzuron or its primary metabolites could be detected in the hydrolysates.

Of the total radioactivity in the straw, 71%-81% was extractable (Table 23). The parent (36%-42% of the TRR) and CPU (26%-29%) were the major residues. Minor metabolites were CPU conjugates (2.5% of the TRR), DFBA conjugates (2.1%), PCA (0.2%) and unknown compounds (5.8%-8.6%). Acid/base hydrolysis released 15% of the TRR from the unextractable residues, with CPU as the major product (10% of the TRR) and DFBA (2.2%), PCA (0.4%) and unknown compounds (2.2%) as minor products.

Table 22. Total radioactive residues expressed as mg/kg diflubenzuron equivalents in rice plants.

Sample	DAT	0.28 kg ai/ha	1.7 kg ai/ha
Immature plant	0	133	755
Immature plant	30	0.90	17
Mature grain	109	0.091	0.66
Mature straw	109	1.0	9.0

Table 23 Distribution of radioactive residues in rice grain and straw as % of TRR.

Sample	Extract	0.28 kg ai/ha	1.7 kg ai/ha
Mature grain	MeOH:H_2O:HAc	21%	26%
	ACN:H_2O:HCl	5.2%	5.0%
	Total extracted	26%	32%
	Bound	51%	62%
Mature straw	ACN	51%	62%
	ACN:H_2O:HAc	16%	16%
	ACN:H_2O:HCl	3.6%	3.4%
	Total extracted	71%	81%
	Bound	18%	17%

Mushrooms (compost and casing treatment). Four boxes (surface area 0.24 m^2) were filled with compost containing *Agaricus bitorquis*. Two contained a compost layer of 17 cm, the other two a layer of 10 cm. Diflubenzuron in which either the difluorobenzoyl moiety was labelled with ^3H (specific activity 20.6 mCi/g; radiochemical purity 99.4%) or the chloroaniline moiety with ^{14}C (specific activity 2.0 mCi/g, radiochemical purity 99.0%) was added as a dispersion in water at a dose of 10 kg ai/ha to the compost of each box on day 1. At casing (day 13) the compost was covered with a 4 cm layer of casing soil and each box received another dose of 10 kg ai/ha. The growth medium of each box was sampled in triplicate at casing and at each of the four flushes of mushrooms (days 32, 38, 46 and 55). Mushrooms from each flush from each box were considered to be one sample. Storage conditions were not reported (Nimmo and De Wilde, 1977; Nimmo, 1986, both non-GLP).

All samples were extracted successively twice with ACN and twice with methanol/water (1:1) and the extracts pooled. In addition, both the homogenized mushrooms and the extracts from mushrooms treated with [^3H]diflubenzuron were steam-distilled. Extracts were analysed by LSC, and unextractable residues by combustion and LSC. Diflubenzuron, CPU, PCA and DFBA were determined by reversed isotope-dilution analysis. The identity of the metabolites was confirmed by TLC (silica gel; two solvent systems) with autoradiographic and UV detection. Reference compounds were diflubenzuron, CPU and PCA.

The recovery of the applied radioactivity and its distribution were not reported. In the growth medium 88%-89% of the TRR (^{14}C) and 92%-96% (^3H) was identified as the parent compound on day 13, the day of the last application (Table 24). From day 32 onwards diflubenzuron was not degraded further and reached mean levels of 56% (^{14}C) and 72% (^3H) of the TRR. There were no significant differences between the levels or composition of the residues in 10 cm and 17 cm compost layers. The main metabolites (days 32-55) from the ^{14}C compound were CPU (25%-38% of the TRR), unidentified extractable residues (<6.5%) and bound residues (9.5%-18%), and from the ^3H compound DFBA (10%-33%), unidentified extractable residues (<7.6%) and bound residues (4.2%-8.2%). PCA was present at <1% of the TRR.

The total residue in the mushrooms expressed as diflubenzuron equivalents was 0.33-1.0 mg/kg (^{14}C) and 3.2-11 mg/kg (^3H) (Table 24). There was most parent compound in the first flush of mushrooms (8.2%-17% of the TRR; day 32) and it decreased to levels at or below the LOQ in subsequent flushes. The main ^{14}C metabolites (days 32-55) were CPU (54%-82% of the TRR), unidentified extractable residues (0%-19%) and bound residues (16%-25%), and the main ^3H metabolites were DFBA (25%-43% of the TRR), unidentified extractable residues (50%-70%) and bound residues (3.0%-5.9%). In the ^{14}C extracts PCA was <1% of the TRR (0.02 mg/kg PCA) at day 32. TLC analysis of ^{14}C extracts revealed diflubenzuron, CPU and spots with Rf = 0, representing polar metabolites (e.g. conjugates). The ^{3}H residue levels were much higher than the ^{14}C and a considerable part of the extracted residue was not identified. Steam distillation of mushrooms and mushroom extracts indicated that 40%-70% of the total recovered ^{3}H residue was tritiated water.

Table 24. Mean distribution of ^{14}C and ^{3}H diflubenzuron residues in mushroom growth medium.

Com-post depth	Day	^{14}C-labelled diflubenzuron					^{3}H labelled diflubenzuron				
		Total ^{14}C mg/kg	Difluben-zuron % of TRR	CPU % of TRR	Unknown % of TRR	Bound % of TRR	Total ^{3}H mg/kg	Difluben-zuron % of TRR	DFBA % of TRR	Unknown % of TRR	Bound % of TRR
17 cm	13	591	88	9.2	0.0	4.3	106	92	3.9	0.3	3.4
	32	33	49	37	0.0	18	41	59	33	0.0	8.2
	38	70	63	30	0.0	9.5	46	72	16	7.1	5.7
	46	44	51	38	0.0	13	54	70	19	4.0	6.9
	55	42	55	25	3.3	16	24	63	22	7.6	7.8
10 cm	13	108	89	5.8	0.9	4.6	161	96	3.0	0.0	4.0
	32	39	58	38	0.0	10	50	77	14	2.3	6.0
	38	39	58	33	0.0	9.7	67	74	19	2.7	4.2
	46	43	58	29	0.7	13	34	80	10	3.4	6.6
	55	33	55	25	6.5	13	48	80	11	4.1	4.7

Unknown: unidentified extracted residues, calculated from total extracted by difference

Table 25. Mean distribution of ^{14}C and ^{3}H diflubenzuron residues in mushrooms.

Layer	Day	^{14}C-labelled diflubenzuron					^{3}H labelled diflubenzuron				
		Total ^{14}C mg/kg	Difluben-zuron % of TRR	CPU % of TRR	Unknown % of TRR	Bound % of TRR	Total ^{3}H mg/kg	Difluben-zuron % of TRR	DFBA % of TRR	Unknown % of TRR	Bound % of TRR
17 cm	32	1.0	17	54	5.8	23	7.2	1.7	43	50	5.6
	38	0.33	nd	64	7.6	25	3.2	2.3	26	65	5.9
	46	0.68	nd	63	19	17	7.0	0.1	25	70	4.8
	55	na	na	na	na	na	11	0.3	35	59	5.6
10 cm	32	0.37	8.2	63	13	16	na	na	na	na	na
	38	na	na	na	na	na	6.1	0.2	38	58	4.1
	46	0.72	nd	82	0	22	6.2	0.2	29	68	3.0
	55	na	na	na	na	na	na	na	na	na	na

na: not analysed
nd: not detected (<0.01 mg/kg diflubenzuron equivalents)
unknown: unidentified extracted residues, calculated from total extracted by difference

Mushrooms (compost or casing treatment). In duplicate experiments compost was seeded with *Agaricus bisporus*, (U1 variety). Diflubenzuron (^{14}C-labelled in both phenyl rings; specific activity 2.16 mCi/g; radiochemical purity 99.3%) was added as a suspension to the compost or at casing at a dose of 50 kg ai/ha; controls received water only. The growth medium was sampled in duplicate at application (day 0 for compost, day 15 at casing), at the harvest of each of three flushes of mushrooms (days 41, 48, 55) and 52 days after the last flush (day 107). Mushrooms from each flush were considered to be one sample. Samples were extracted within 24 hours. Growth medium samples were extracted successively with ACN and methanol and the extracts pooled. Mushrooms were extracted with 0.5 M HCl/ACN (1:1) and ACN and the pooled extracts were extracted with diethyl ether (Thus and Van der Laan-Straathof, 1993b, GLP).

Extracts were analysed by LSC and HPLC-LSC (Nucleosil C-8); unextractable residues were analysed by combustion and LSC. The identity of the metabolites was confirmed by TLC (silica gel;

one solvent system) with autoradiographic detection. Reference compounds were diflubenzuron, CPU, DFBA, DFBAM, PCAA, 2-OH-DFB and 3-OH-DFB.

When untreated mushrooms were spiked with diflubenzuron, DFBA, CPU and PCA at levels of 0.0005-0.002 mg/kg diflubenzuron equivalents, the mean extraction efficiency was >95% for diflubenzuron, 71% for DFBA, 74% for CPU and 24% for PCA. With higher spiking levels, the mean extraction efficiencies were 82% and 48% at 1.4 and 2.8 mg/kg diflubenzuron equivalents respectively for DFBA, 100% and 62% at 0.10 and 0.20 mg/kg diflubenzuron equivalents respectively for CPU, and 45% and 24% at 0.15 and 0.30 mg/kg for PCA.

The recovery and distribution of the total applied radioactivity were not reported. In the growth medium 93%-101% of the total recovered radioactivity from compost treatment and 67%-91% from casing treatment was identified as the parent compound during the time the mushrooms were grown up to day 55 (Table 26). Diflubenzuron applied to the casing was metabolized more rapidly than when applied to the compost: the main metabolites from the casing treatment up to day 55 were DFBA (0.03%-8.0% of the TRR), CPU (0.02%-10%) and bound residues (2.0%-21%). At the end of the experiment (day 107; casing treatment) the radioactivity was distributed between the parent (23%), DFBA (32%) and bound residues (36%).

The total ^{14}C residue in the mushrooms expressed as diflubenzuron equivalents was 0.12-0.35 mg/kg (mean 0.21) from the compost treatment and 5.9-9.4 mg/kg (mean 8.0) from the casing treatment. There were no significant differences in the levels or composition of the residues between the three flushes. The composition of the ^{14}C residue in the mushrooms is shown in Table 27. The main part of the residue from both treatments consisted of DFBA (81%-88% of the TRR).

Table 26. Mean composition of ^{14}C residues in mushroom growth medium, expressed as % of TRR (n = 4).

Compound	Compost treatment					Casing treatment				
day	0	41	48	55	107	15	41	48	55	107
DAT	0	41	48	55	107	0	26	33	40	92
diflubenzuron (parent)	93	95	94	101	89	91	81	82	67	23
DFBA	<0.05	1.1	1.7	0.83	1.7	0.03	5.8	6.2	8.0	32
CPU	<0.05	1.4	2.2	0.90	0.60	0.02	10	9.5	10	5.4
PCA	<0.05	0.35	0.20	<0.05	0.06	<0.01	0.23	0.12	0.09	0.09
bound residues	3.6	5.8	7.9	5.4	7.1	2.0	7.2	9.4	21	36
Total	97	103	106	108	98	93	104	107	107	97

TRR: total recovered radioactivity
DAT: days after last treatment

Table 27. Mean composition of ^{14}C residues in mushrooms from three flushes (days 41, 48, 55).

Compound and fraction	Compost treatment		Casing treatment	
	mg/kg equivalents	% of TRR	mg/kg equivalents	% of TRR
Total ^{14}C (extracted plus bound)	0.21	100	8.0	100
parent (diflubenzuron); ACN extract	≤0.004	1.9	0.04	0.5
DFBA; ACN extract [1]	0.17	81	7.1	88
CPU; diethyl ether extract	≤0.0008	0.4	0.06	0.7
PCA; diethyl ether extract [1]	≤0.002	1.0	0.05	0.6
OH-DFB; ACN extract	≤0.01	4.8	0.02	0.2
bound residues	0.02	9.5	0.27	3.4

[1] Results were corrected for recovery: 82% for DFBA and 24% for PCA

Tomato and broad beans (CPU uptake from nutrient solution). Tomato and broad bean plants were grown for 6 weeks and 10 days respectively in a nutrient solution containing CPU ^{14}C-labelled in the phenyl ring (specific activity 5.1 mCi/g; radiochemical purity not reported) at a concentration of 0.7 and 1.0 mg/l respectively. Tomato plants were cut 10 cm above the roots. In another experiment the broad bean plants were grown in a 0.5 mg/l solution for 3 days and than transferred to a blank nutrient solution for another 3 days. Solutions were aerated during the experiment (Van den Berg, 1978a, non-GLP).

The CPU solution and exudates (xylem sap) from the tomato root/stem systems were analysed after 0, 1, 2, 3 and 6 days by LSC. Bean shoots and roots from the 1.0 mg/l experiment were analysed separately after 3 and 7 days by extraction with hot ACN and hot 50% methanol. Bean shoots and roots from the 0.5 mg/l experiment were extracted with ACN (first at room temperature then by reflux). Extracts and unextractable residues were analysed by combustion and LSC. Extracts were characterized by TLC (silica gel; 1 solvent system). PCA was determined by reversed isotope dilution analysis.

The CPU concentration in a solution without the tomato root/stem system was stable for 6 days (results were not reported). When the solution was aerated the CPU concentration increased from 0.69 mg/l at day 0 to 0.84 mg/l at day 6 owing to the evaporation of water.

At day 6 the CPU concentration in the aerated solution with the tomato root/stem system had decreased to 0.50 mg/l (60% of the aerated control solution) and the concentration in the exudates had increased 0.36 mg/l (43% of aerated control solution).

In the 1.0 mg/l broad bean experiment 94% ± 4.9% RSD_r and 91% ± 5.3% of the applied radioactivity was recovered after 4 and 7 days respectively. After 4 days 27% ± 20% RSD_r of the applied radioactivity was taken up by the plant: 22% ± 29% RSD_r in the shoots and 5.8% ± 18% in the roots. After 7 days 59% ± 25% was taken up by the plant with 49% ± 26% in the shoots and 9.5% ± 19% in the roots. In the shoots 97% of the TRR was extractable after both 4 and 7 days; in the roots 61% and 71% of the TRR was extractable after 4 and 7 days respectively. TLC analysis showed that 95% of the extractable residue consisted of CPU; the remainder stayed at the origin of the TLC plate and was probably conjugated material.

In the 0.5 mg/l broad bean experiment 94% ± 2.0% and 97% ± 3.5% of the applied radioactivity was recovered after 3 and 6 days respectively. After 3 days 24% ± 18% of the applied radioactivity was taken up by the plant, with 19% ± 21% in the shoots and 4.3% ± 7.0% in the roots. After another 3 days in blank solution 17% ± 12% of the applied radioactivity remained in the plant: 16% ± 12% in the shoots and 0.9% ± 37% in the roots. In the shoots 96% and 92% of the TRR and in the roots 90% and 60% was extractable after 3 and 6 days respectively. The nutrient solution contained 0.24% (1.3 µg/l) PCA at day 0 and 0.11% (0.7 µg/l) at day 3. No PCA could be detected in the extracts of shoots or roots from days 3 and 6.

It was concluded that CPU is rapidly taken up by the roots and transported via the xylem to the leaves. It accumulates in the leaves and its metabolism is very slow.

Tomato (DFBA uptake from nutrient solution). Tomato plants were grown in a nutrient solution for 6 weeks and cut 10 cm above the roots. The root/stem systems were placed for 6 days in a solution containing DFBA ^{14}C-labelled in the carboxylic group (specific activity 2.0 mCi/g; radiochemical purity not reported) at a concentration of 1.8 mg/l. All solutions were aerated. In another experiment, the root/stem systems were placed in a desiccator for 6 days and the volatiles were trapped in monoethanolamine/2-methoxyethanol (1:4). DFBA solution and exudates (xylem sap) were analysed after 1, 2, 3 and 6 days by LSC (Van den Berg, 1978b, non-GLP).

DFBA in a solution without the root/stem system was stable for 6 days; when the solution was aerated the DFBA concentration increased slightly owing to the evaporation of water (results were not reported). 100% of the applied radioactivity was recovered from the solution in the desiccator and 1.7% from the trap.

At day 6 the DFBA concentration in the aerated root/stem system had decreased to 0.87 mg/l (49% of the original concentration), the concentration in the exudates was 0.03 mg/l (1.7% of the original solution concentration) and 50% of the radioactivity was missing. From the plants in the desiccator 33% of the applied radioactivity was recovered from the solution, 35% from the trap and 33% was missing.

It was concluded that DFBA is decarboxylated rapidly by tomato roots and the xylem sap contains very little DFBA.

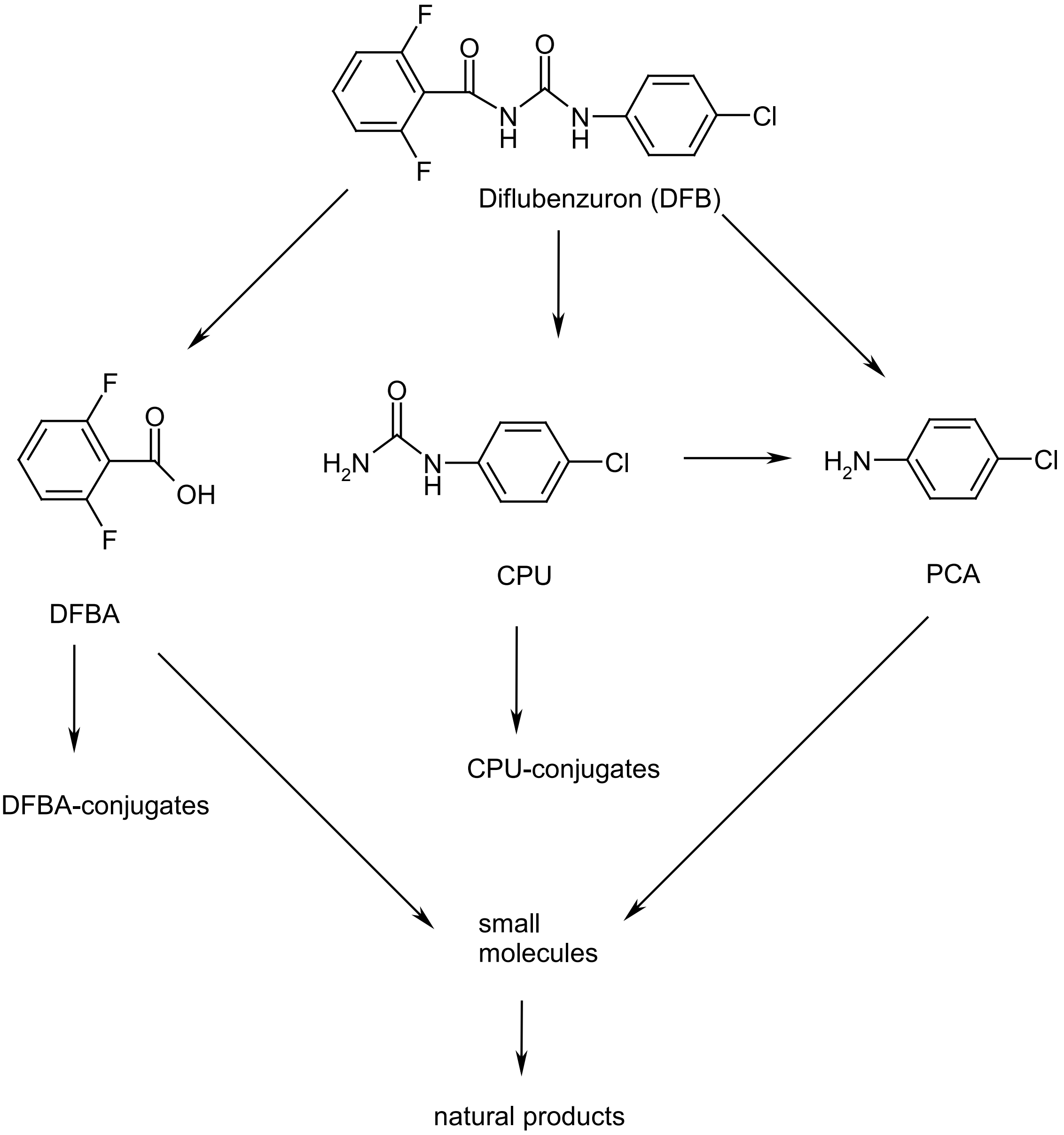


Figure 2. Proposed metabolic pathways of diflubenzuron in rice and mushrooms. In other crops there was little metabolism

Environmental fate in soil

The Meeting received information on the fate of diflubenzuron in soil: degradation in laboratory and field studies, photodegradation on soil layers, soil adsorption and leaching and rotational crop studies (greenhouse and field).

Degradation. A laboratory transformation study with ring-labelled [^{14}C]diflubenzuron (radiochemical purity 99.0%, specific activity 9.91 mCi/g) was conducted in a sandy loam soil according to EPA guideline 162-1 (Walstra and Joustra, 1990, GLP). The soil was collected at Udenhout (The Netherlands), passed through a 2 mm sieve and the water content was adjusted to pF 2.7. The flasks with soil were pre-incubated at 24°C in the dark for 10 days to restore the microbial activity. Soils (43.1 g) were dosed with diflubenzuron at a rate of 0.69 mg ai/kg in a suspension with a 7:3 (w/w) mixture of sodium lignin sulfonate and sodium dialkylnaphthalene sulfonate; the ratio of diflubenzuron:sulfonate mixture was 2.2:1.5. Incubation was in the dark for 21 days with CO_2 traps (KOH). Three flasks were sampled at 0, 6, 24, and 48 hours and 4, 7, 10, 14, and 21 days. Extraction was with ACN and methanol. Analysis was by LSC and HPLC (two different systems). The bound residues were determined by combustion and LSC. The analytical recovery was determined by spiking blank soil extracts with amounts of diflubenzuron ranging from 1 to 100% of the initial amount in the samples. The mean recovery of diflubenzuron was 99%.

The total recovery of ^{14}C ranged from 94% to 101% of the applied diflubenzuron. The impurities in the diflubenzuron added to the soil samples were <0.2%. The amount of CO_2 increased gradually to 26% and bound residues to 37% of the applied diflubenzuron after 21 days. Four metabolites were identified; all except CPU were <10% of the TRR. CPU increased to a maximum of 31% of the applied diflubenzuron after 7 days and decreased to 25% by the end of the study (day 21). Transformation followed first order kinetics. The authors calculated a half-life for diflubenzuron of 50 hours, using the first 6 data points (7 days; r^2=0.995), and a half-life for CPU of 43 days (extrapolated value), using time points from days 7 to 21, r^2=0.96.

No information was given on the soil history. It was not clear whether soils were stored before use. The disperging agents used are constituents of the Dimilin WP formulation. The results are considered reliable.

The aerobic degradation of a 1:1 mixture of [^{14}C]chlorophenyl-labelled diflubenzuron (radiochemical purity ≥99.4%, specific activity 2.35 mCi/mmole) and [^{3}H]-benzoyl-labelled diflubenzuron (radiochemical purity ≥99.0 %, specific activity 22.9 mCi/mmole) was determined in 3 soils: Tollebeek sandy loam, Schinnen silt loam and 's-Graveland sand, in The Netherlands (Nimmo and De Wilde, 1975, non-GLP). In the sandy loam, the anaerobic and sterile degradation of ^{14}C-labelled diflubenzuron was also determined. Dispersions had to be used because of the low solubility of diflubenzuron. A recrystallised mixture (1:1) of milled ^{14}C- and ^{3}H-labelled diflubenzuron was supplemented with 30% (w/w) of a 7:3 mixture of Polyfon H dispersing agent and Sellogen HR wetting agent. The preparation was mechanically milled in a conical glass mortar until the desired size of the particles had been reached, according to microscopic examination (≤5 µm; mean 2 µm). Sieved soil samples (100 g) were moistened to 40% of field capacity in 300 ml open Erlenmeyer flasks. Triplicate flasks were fortified at a rate of 1 mg ai/kg with diflubenzuron suspension.

The aerobic incubation was for 0, 14, 28, 56, 98, 182 and 364 days (182 days for sand) in the dark in polythene boxes. The inner walls of the boxes were covered with wet filter paper. Anaerobic incubation was for 0, 14, 28, 56, 98 and 182 days in closed flasks in a nitrogen atmosphere. Sterile incubation was for 28 days and 1 year. The contents of triplicate flasks were extracted separately by homogenizing the soil with 150 ml ACN twice, followed by 2 or 3 reflux extractions with methanol/water (1:1). The total extractable radioactivity was determined by LSC of pooled extracts.

The bound residues were determined by combustion and LSC. Diflubenzuron and CPU were identified and quantified by reversed isotope-dilution analysis, TLC and MS.

In the aerobic incubations the total ^{14}C recovery (total extractable radioactivity plus bound residue) decreased to 53% in sandy loam, 60% in silty loam and 65% in sand at termination (364 days for first two soils, 182 days for sand). The total ^{3}H recovery was 8, 10 and 20% respectively, extractable ^{14}C 5, 12 and 16%, and extractable ^{3}H 2, 2 and 11%. After 14 days, 4-5% of the applied radioactivity was identified as diflubenzuron; the transformation rate decreased when <5% of the diflubenzuron was left. After 28 days 2% diflubenzuron remained, after 98 days <1% (below the LOQ). CPU was rapidly formed, after 28 days 43, 55 and 43% of the applied radioactivity was recovered from sandy loam, silty loam and sand respectively, and at termination 5, 12 and 31%. The bound residues reached maxima of 50% in sandy loam after 182 days (21% at day 98, 48% after 364 days), 48% in silty loam after 364 days (32% at 98 days) and 49% in sand after 182 days (14% at 98 days). ^{3}H bound residues were 4-7% after 98 days and 6-9% at the end of the experiment. Maximum levels were 9, 13 and 9% at days 14, 14 and 182, and the estimated half-lives were 3, 4 and ≤4 days in loamy sand, silty loam and sand respectively.

According to the authors DFBA is also formed, but is very unstable. Circumstantial evidence for rapid transformation of DFBA is given by the fact that only a minor part of the losses of extractable ^{3}H can be explained by bound residue formation. Under anaerobic conditions most of the initially applied ^{3}H was recovered, indicating the formation of a volatile transformation product of DFBA that may have escaped from the open flasks during the aerobic experiments. In a separate large-scale experiment with the clay hydrosoil (no details) the soil water layer was found to contain 70% of initially applied ^{3}H: 10% as DFBA and 60% incorporated into water.

In the anaerobic incubations the total recovery of ^{14}C and ^{3}H was 54% and 94% respectively after 182 days, and extractable ^{14}C and ^{3}H to 15 and 77%. Diflubenzuron decreased to 4% of the applied radioactivity after 14 days; its transformation rate decreased when <10% was left. After 28 and 182 days, 40 and 15% CPU was formed. ^{14}C and ^{3}H bound residues were 31 and 7% respectively after 98 days and increased to 39 and 17% at termination (182 days). In the sterile incubations there was practically no degradation after 28 days: the total extractable radioactivity was 95%, with 94% remaining as diflubenzuron. The bound residue amounted to 5%. After 1 year 5% of the diflubenzuron was still recovered from the soil. In a concurrent non-sterile control run the extractable radioactivity, diflubenzuron and bound residue amounted to 43, 2 and 27% respectively after 28 days.

In the aerobic incubations the analysis points were not properly chosen: 4-5% diflubenzuron remained after 14 days, so the estimated half-life could only be <14 days. In the anaerobic incubations soils were too dry. There was no information on soil history, and it was not clear whether the soils were stored before use. The disperging agents used are constituents of the Dimilin WP formulation. Incubations were open and volatiles were not trapped, which may have caused low total recovery. The results are not considered reliable.

The aerobic degradation of [^{14}C]chlorophenyl-labelled diflubenzuron (radiochemical purity ≥99.4%, specific activity 2.35 mCi/mmole) was determined in 2 German BBA standard soils: sand 2.1 (Hatzenbühl) and loamy sand 2.2 (Hatzenbühl) (Nimmo and De Wilde, 1975, non-GLP). Methods were as described above. The aerobic incubation of duplicate flasks was for 0, 3.5, 10.5, 17.5, 31.5, 66.5 and 112 days in the dark at a fortification rate of 1 mg/kg, with a mean size of dispersed particles of 2 μm.

The total recovery of ^{14}C (total extractable radioactivity and bound residue) decreased to 69% and 56% at termination in sand and sandy loam respectively, with extractable radioactivity 29% and 22%. In loamy sand 65% of the applied radioactivity was present after 3.5 days, and in sand 35%. The degradation rate decreased when <10% diflubenzuron was left. After 112 days diflubenzuron was below the LOQ (<1%). CPU was rapidly formed with maxima of 61 and 68% after 10.5 and 17.5 days in sand and loamy sand respectively. The DT-90 was not reached: 12 and 25% remained at termination. The bound residues reached maxima of 21% after 17.5 days in sand, and 21% after 66.5 days in sandy loam. The authors reported half-lives of 3 and 4 days in sand and loamy sand respectively. The transformation of diflubenzuron and CPU followed first-order kinetics.

The soil incubations were too dry. There was no information on soil history, and German BBA soils sampled before 1982 were probably stored too dry. The disperging agents used are constituents of the Dimilin WP formulation. Incubations were open and volatiles not trapped, which may have caused low total recovery. The half-life in sand was <4 days. The results are not considered reliable.

The aerobic degradation of milled carbonyl-labelled [^{14}C]diflubenzuron (radiochemical purity ≥99.0%, specific activity 0.32 mCi/mmole) was determined in 3 Dutch soils: Ophemert clay, Boskoop peat, and 's-Graveland sand (Nimmo and De Wilde, 1975, non-GLP) by the methods described above at a fortification rate of 1 mg/kg. The mean size of the dispersed particles was 10 µm (range 0-50 µm). Aerobic incubation of duplicate flasks was for 0, 14, 21, 42, 84, 147 and 273 days in the dark. The bound residue was determined after 147 and 273 days only.

The transformation patterns were very similar in all three soils: the total recovery decreased to 42%, 56% and 35% respectively at termination and the extractable radioactivity to 40%, 49% and 32%. The lost activity was largely recovered as $^{14}CO_2$ in an additional experiment with clay (no further details). The transformation of diflubenzuron did not follow first order kinetics; 42%, 49% and 33% remained at termination. Bound residues were 2-7% at days 147 and 273. According to the authors the relatively long half-life results directly from differences in particle size, the apparent half-life being governed by the rate of dissolution as well as by the true rate of degradation. The rate of dissolution of particles is linearly correlated with their surface area. The authors state that the half-life of diflubenzuron applied to soil as 2 µm particles is similar to that when a true solution is used. On the assumption that the particles are spherical a mean difference in rates of dissolution of a factor 25 would be expected between 2 and 10 µm particles.

Again the soils were too dry, with no information on history. The organic matter content of peat was too high. The mean size of the dispersed diflubenzuron particles is 5 times that in commercially available formulations, and this is assumed to have caused slow transformation. The results are not considered reliable.

The anaerobic degradation of a 1:1 mixture of chlorophenyl-labelled [^{14}C]diflubenzuron (radiochemical purity ≥99.4%, specific activity 2.35 mCi/mmole) and benzoyl-labelled [^{3}H]diflubenzuron (radiochemical purity ≥99.0%, specific activity 22.9 mCi/mmole) was determined in 3 Dutch soils: Nederhorst den Berg clay and peat, and Tollebeek sandy loam soil (Nimmo and De Wilde, 1975, non-GLP) at a fortification rate of 1 mg/kg. The mean size of the dispersed particles was 2 µm. Anaerobic incubation of triplicate flasks was for 0, 14, 28, 56, 98 and 182 days in the dark with 300 ml ditch water added to 100 g soil.

The total ^{14}C recovery decreased to 51%, 52% and 90% at termination in the clay, peat and sandy loam respectively. ^{3}H recovery varied between 88% and 101%. Extractable ^{14}C decreased to 14%, 35% and 32% and extractable ^{3}H to 75% in peat and 82%-98% in the other soils. Transformation generally followed first order kinetics; 16%, 5% and 3% of the applied radioactivity was recovered as diflubenzuron after 14 days. The degradation rate decreased when 10% of the diflubenzuron was left. ^{14}C bound residues increased to 37%, 17% and 58% after 182 days respectively. ^{3}H bound residues in peat increased within 98 days to 22% and varied between 2 and 7% in the other soils.

The organic matter content of the peat was too high. There was no information on soil history and it was not clear whether the soils were stored before use. The time points were not properly chosen, since there was >50% degradation within the first time interval. The results are not considered reliable.

The anaerobic degradation of ring-labelled [^{3}H]DFBAM (radiochemical purity ≥99%, specific activity 2.7 mCi/mmole) and production of its transformation product DFBA was determined in Nederhorst den Berg clay flooded soil from The Netherlands (Nimmo and De Wilde, 1975, non-GLP). Sieved soil samples (100 g) were moistened to 40% of field capacity in 500 ml Erlenmeyer flasks and fortified at a rate of 1 mg/kg with DFBAM solution. The soil was then covered with 300 ml ditch water and the flasks closed with ground glass stoppers. Incubation of triplicate flasks was for 0, 7, 18, 28, 42 and 77 days in the dark. Extraction was by homogenizing with ACN and refluxing with water/methanol. The total extractable residue was determined by LSC of pooled extracts and the bound residue by combustion and LSC. DFBAM and DFBA were quantified by reversed isotope dilution analysis, TLC and MS.

The total recovery of radioactivity decreased to 79% by day 77. The bound residue increased to about 10% by day 18 and remained stable thereafter. The total extractable radioactivity decreased steadily to 69% by day 77. It was initially entirely associated with DFBAM, which rapidly decreased, following first order kinetics, with <1% remaining by day 77. The major degradation product DFBA reached a maximum of 40% by day 28 and then decreased to 13% by day 77.

The total recovery was <80%. The extractable radioactivity was only partially accounted for as DFBA and DFBAM. It is not certain whether anaerobic conditions were established at the start; the system was probably aerobic for a period. There was no explanation of the remaining extractable activity. The results are not considered reliable.

The aerobic degradation of uniformly ring-labelled [^{14}C]CPU (radiochemical purity 99%; specific activity 0.86 mCi/mmole) was determined in Tollebeek sandy loam in The Netherlands (Nimmo and De Wilde, 1975, non-GLP). Sieved soil samples (100 g) were moistened to 40% of field capacity in 300 ml open Erlenmeyer flasks and fortified at a rate of 1 mg/kg with CPU solution. Incubation was for 0, 14, 28, 56, 112, 182, 273 and 364 days in the dark in polythene boxes. The inner walls of the boxes were covered with wet filter paper. The effect of sterilization was examined in autoclaved soil, from which one sample was taken after 273 days. Extraction and analysis were as above. CPU was quantified by reversed isotope dilution analysis, TLC and MS.

The total recovery decreased to 75% by day 56, but then increased to 97% and 98% by days 112 and 182, and decreased to 67% by day 364. Bound residues steadily increased to 53% after 98 days, reached a maximum of 79% by day 182 and decreased thereafter to 60% by day 364. The extractable radioactive residue consisted mainly of CPU and minor amounts of an unknown product. PCA was not found in the extracts, in which the radioactive residue had decreased to 7% by day 364. The transformation of CPU followed first order kinetics with 5% remaining by day 364. The distribution did not change after day 273.

In sterilized soil only 15% of bound residue was formed. The total recovery of radioactivity amounted to 98% by day 273. Transformation during this period was hardly significant: the total extractable radioactivity was 83% with 81% identified as CPU.

The soil was too dry (pF[1] 3.7) and the recovery was <80% at several times. The results are not considered reliable.

The transformation of ring- and carbonyl-labelled [^{14}C]CPU (radiochemical purity 96.0%, specific activity 5.4 mCi/g, and purity 97.5%, specific activity 9.9 mCi/g) was investigated under aerobic, sterile, and anaerobic conditions in a Dutch soil (Tollebeek loam), its hydrosoil, a silty clay loam hydrosoil and a peat hydrosoil (Nimmo and Willems, 1984, non-GLP). A stock solution of CPU in acetone was diluted with water and triplicate soil samples (50 g at 40% of field capacity) in 200 ml biometer flasks were fortified at a rate of 1 mg/kg by adding 2 ml of CPU solution. Incubation was in the dark at 24°C or 14°C for the aerobic treatment. Anaerobic conditions for the hydrosoils were met

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[1] -log (SWP). SWP is soil water potential, a function of moisture content and texture.

by adding tap water to 40 g dry soil to a total volume of 200 ml. Sterile conditions were established by autoclaving twice. Triplicate soil samples were taken at 0, 28, 56, 112, 224 or 231, 336 and 448 days; experiments lasted 32 to 64 weeks. $^{14}CO_2$ was trapped in KOH and measured by LSC. Extracted soil was homogenized twice with 70 ml ACN, then refluxed twice with 70 ml methanol (time not reported). The total ^{14}C activity was assayed by LSC. Identification of CPU and PCA in pooled extracts was by TLC, with quantification by determination of specific activity in recrystallized silica gel column fractions. Bound residues were determined by combustion and LSC.

In the first two incubations (24°C) the total recovery decreased from 100% to 82%-102% of the applied radioactivity. Extractable residues steadily decreased and consisted entirely of CPU. The bound residues of ring-labelled CPU reached a maximum of 72% after 224 days and then decreased to 57% after 448 days. CO_2 production from ring-labelled CPU was 19% and 14% after 448 and 231 days respectively. The bound residues from carbonyl-labelled CPU reached a maximum of 24% after 28 days and then decreased to 11% after 448 days (the end of the study). CO_2 production increased to 87% after 448 days.

In the third and fourth incubations at 14 and 24°C the total recoveries ranged from 95% to 106%. Maximum bound residues were 62% of the applied radioactivity at 14°C and 68% at 24°C after 112 days; after 231 days they were 61% and 68%. $^{14}CO_2$ reached 6% and 13% at 14 and 24°C after 112 days, and 12% and 28% at the end of the experiment.

At the end of the sterile soil incubation 85% of the applied radioactivity was recovered. Only 15% could not be extracted and may have consisted of adsorbed CPU or transformation products (no further details).

In the anaerobic incubations the total extractable and bound residues accounted for a maximum of 109% and amounted to 61%-101% at termination. Extractable activity decreased steadily and was almost entirely due to CPU. The transformation kinetics of ring- and carbonyl-labelled CPU were similar and approached first order; 21%-47% remained as CPU after 236 and 448 days. The bound residues reached plateaux of 30-60% after 112 days.

In the aerobic incubation with loam hydrosoil the total recovery was 83-104% of the applied radioactivity. Bound residues reached a maximum of 65% after 112 days and were 62% at the end of the experiment after 336 days. $^{14}CO_2$ reached 8% after 112 days and increased to 17% at 336 days.

The CEC of the soils was not reported. The loam soil was much too dry (pF 4). The time between the first and second samples was 4 weeks, when the transformation was about 25%. Transformation in the anaerobic incubations at 24°C did not follow first order kinetics; a significant discontinuity was found between 120 and 130 days. The zero time value is given only for the first two incubations: for all the other incubations it was calculated by linear regression from the other time points. The zero time value for the 14°C incubation was 83%. The results are not considered reliable.

Transformation of ring-labelled [^{14}C]DFBA (radiochemical purity 98.2%, specific activity 10.2 mCi/g) was determined under aerobic conditions in two Dutch soils (Tollebeek loam and Heino sand). 3 ml of a stock 15 mg/l solution of [^{14}C]DFBA in water was added to pre-incubated soil samples (40 g dw). After 0, 1, 2, 4, 8, 16, and 32 days, triplicate samples were sequentially extracted by shaking with 75 ml 1 M NaOH, stirring with 75 ml methanol/water (1:1) and refluxing with the same methanol/water mixture. Radioactivity in the extracts was counted by LSC, and pooled extracts were analysed by HPLC. $^{14}CO_2$ was trapped in KOH and counted by LSC. Soil-bound residues were determined by combustion and LSC (Nimmo *et al.*, 1990, non-GLP).

The total recovery from loam was between 97% and 102% of the applied radioactivity. Extractable radioactivity steadily decreased from 100% of the applied radioactivity on day 0 to 32% on day 32, and DFBA from 98% to 27%. Bound residues increased to 37% of the applied radioactivity, and CO_2 to 28%, after 32 days. The authors estimated a half-life of about 12 days.

The total recovery from sand was between 96% and 103% of the applied radioactivity. Extractable radioactivity steadily decreased from 97% of the applied radioactivity on day 0 to 11% on day 32 and DFBA from 96% to 2%. Bound residues increased to 33% and CO_2 to 52%. The half-life was estimated to be about 9 days.

From the difference between the total extractable residues and DFBA, the authors concluded that <10% of other products had been formed.

The CEC of the soils was not reported. Pre-incubation conditions were not given, the soils were probably too dry but this seems not to have influenced degradation. The results are considered reliable.

Field dissipation. Diflubenzuron was applied 3 times as a WP 250 formulation to a plot of citrus trees in Madera, California, USA at a rate of 0.35 kg ai/ha per application at intervals of two months in May–October, 1989 (Ver Hey, 1991a, GLP). The treated plot (133 m^2) consisted of five sub-plots. Soil samples were collected before the first application, then 0, 1, 3, 7, 14 and 28 days after the first application, 0, 1, 3, 7, 14 and 28 days after the second and 0, 1, 3, 7, 14, 30, 60, 154, 180, 270, 325 and 360 days after the third. Soil cores were divided into 15 cm segments to a depth of 90 cm and three composite samples of each segment from each of the five sub-plots were analysed.

Average soil temperatures of the upper 15 cm of the soil ranged from 23ºC in winter to 58ºC in summer, and average air temperatures from 2ºC to 66ºC. Monthly average rainfall ranged from 0 to 4.4 mm. The total amount of irrigation water per plot was 119.3 mm. The 0-15 cm layer was sandy loam, pH 6.8, om 0.4%, clay 10%, CEC 27 mmol/kg.

Analysis of diflubenzuron spray solutions indicated that the spray contained 104%, 102% and 96% of the expected concentration (0.025 kg ai/hl) for the first, second and third applications respectively. Data from petri dishes placed on the ground indicated that the average amounts of formulation reaching the ground were 83%, 63% and 34% of the amount applied (0.35 kg ai/ha) in the three applications.

Soil samples were stored at –20°C for 2 days to 5 months and stability for 469 days was demonstrated. Samples were thawed and passed through a 2 mm mesh soil screen. Diflubenzuron was determined by HPLC-UV method RES026, CPU by GC-ECD method RES025 and DFBA by GC-ECD method RES031. All results were corrected for soil moisture content and concurrent method recovery (67%-134% for diflubenzuron, 41%-110% for CPU, and 74%-123% for DFBA), but not for the average recoveries from spiked field samples (102% for diflubenzuron, 104% for CPU and 102% for DFBA) nor for interferences (<0.01 mg/kg). Because of low precision (RSD_r >20% at 0.01-0.02 mg/kg), CPU results are not considered valid.

Pre-treatment soil samples and an irrigation water sample showed no residues of any of the test substances. Diflubenzuron was found in the 0-15 cm layer up to 180 days after the last treatment and at 0.03 mg/kg in the 15-30 cm layer in three samples at day 270. CPU and, at low levels, DFBA were found in the 0-15 cm layer from 7 days after the first treatment. No residues above the LOQ (0.01 mg/kg) were found in deeper layers. The results are summarized in Table 28.

Recalculation of the half-life of diflubenzuron by the reviewer using linear regression on log concentrations after the third application, gave a value of 78 days (r^2=0.85), the value reported by the author. As degradation products were formed in small amounts, it was not possible to calculate half-lives for DFBA or CPU.

Table 28. Soil residues, average concentrations in the 0-15 cm layer on a dry weight basis.

Application.	Days after treatment	Diflubenzuron (mg/kg)	CPU (mg/kg)	DFBA (mg/kg)

Application.	Days after treatment	Diflubenzuron (mg/kg)	CPU (mg/kg)	DFBA (mg/kg)
1st spray	0	0.13	<0.01	<0.01
	1	0.14	<0.01	<0.01
	3	0.34	<0.01	<0.01
	7	0.12	0.01	0.01
	14	0.12	<0.01	0.01
	28	0.09	0.01	0.01
2nd spray	0	0.21	0.01	0.01
	1	0.19	0.02	0.01
	3	0.19	0.01	0.01
	7	0.12	0.02	0.01
	14	0.13	0.02	0.01
	28	0.04	0.01	0.01
3rd spray	0	0.09	<0.01	<0.01
	1	0.15	<0.01	<0.01
	3	0.08	0.01	<0.01
	7	0.10	<0.01	<0.01
	14	0.11	<0.01	0.01
	30	0.06	<0.01	0.01
	60	0.04	<0.01	0.01
	154	0.03	<0.01	<0.01
	180	0.02	<0.01	<0.01
	270	<0.01	<0.01	<0.01
	325	<0.01	<0.01	<0.01
	360	<0.01	<0.01	<0.01

A field dissipation study was carried out with diflubenzuron applied as WP 250 formulation to a plot of citrus trees in Oviedo, Florida, USA (Kramer, 1991, GLP). Three applications were made at a rate of 0.35 kg ai/ha per application at intervals of two months in June–October, 1989. The treated plot (575 m^2) consisted of five sub-plots. Soil samples were collected before and 0, 1, 3, 7, 14 and 28 days after the first application, 0, 1, 3, 7, 14 and 28 days after the second and 0, 1, 3, 7, 14, 28, 60, 92, 179, and 269 days after the third. Soil cores were divided into 15 cm segments to a depth of 90 cm and three composite samples were prepared from each of the five sub-plots as above.

Average soil temperatures for the upper 15 cm of the soil ranged from 23ºC in winter to 54ºC in summer and average air temperatures from 10ºC to 60.5ºC. Monthly average rainfall ranged from 1 to 17 mm. The total amount of irrigation water per plot was 45 mm. The 0-15 cm soil layer was sand, pH 5.2, om 1.3%, clay 5%, CEC 29 mmol/kg.

Analysis of diflubenzuron spray solutions indicated that the spray contained 130%, 110% and 94% of the expected concentration (0.0075 kg ai/hl) for the first, second and third applications respectively. The average amounts of formulation reaching the ground were 54% of the amount applied (0.35 kg ai/ha) for each application.

Soil samples were stored at –20°C for 1-13 months and stability for 480 days was demonstrated. Analytical methods were as above. Those samples that showed an interference peak close to diflubenzuron were reanalysed by GC-ECD method L. All results were corrected for soil moisture content, concurrent method recoveries (73%-119%) and average field spike recoveries (99% for diflubenzuron, 72% for CPU and 88% for DFBA), but not for interferences (max 0.098 mg/kg for diflubenzuron by GLC, <0.01 mg/kg for CPU and DFBA). Because of interferences, results below 0.3 mg/kg diflubenzuron are not considered valid.

Pre-treatment soil and irrigation samples showed no residues of any of the test substances. The results are summarized in Table 29. Diflubenzuron was found in the 15-30 cm layer 0, 1 and 7 days after the first application and on the day of the third application. DFBA was found in the 0-15 cm layer 7 and 14 days after the first treatment and 1-14 days after the second. None was found after the third application, and no residues above the LOQ were found in deeper layers. No CPU was found.

The reviewer recalculated the half-life of diflubenzuron after the third application as above, giving a half-life of 13 days (r^2=0.75), as reported by the author. It was not possible to calculate half-lives for DFBA or CPU.

Because of interferences in the control soil the half-life is not considered reliable.

Table 29. Soil residues, average concentrations in the 0-15 cm layer on a dry weight basis.

Application	Days after treatment	Diflubenzuron (mg/kg)	DFBA (mg/kg)	CPU (mg/kg)
1st spray	0	0.079 (0-30cm)	<0.01	<0.01
	1	0.064 (0-30cm)	<0.01	<0.01
	3	0.053	<0.01	<0.01
	7	0.062 (0-30cm)	0.024	<0.01
	14	0.020	0.018	<0.01
	28	0.027	<0.01	<0.01
2nd spray	0	0.081	<0.01	<0.01
	1	0.083	0.014	<0.01
	3	0.046	0.020	<0.01
	7	0.016	0.043	<0.01
	14	0.015	0.024	<0.01
	28	<0.01	<0.01	<0.01
3rd spray	0	0.094 (0-30 cm)	<0.01	<0.01
	1	0.090	<0.01	<0.01
	3	0.049	<0.01	<0.01
	7	0.034	<0.01	<0.01
	14	0.024	<0.01	<0.01
	28	0.021	<0.01	<0.01
	60	<0.01	<0.01	<0.01
	92	<0.01	<0.01	<0.01

A similar study was carried out with the WP 250 formulation on a plot of apple trees in Phelps, New York, USA, according to EPA guideline, subdivision N, 164-1 (Kramer, 1990, GLP). Three applications were made at 0.28 kg ai/ha at intervals of 21 days in June-July 1989. The treated plot (0.43 ha) consisted of five sub-plots of three rows of five trees. Soil samples were collected before and 0, 1, 3, 7, 14, and 21 days after the first application, 1, 3, 8, 14, and 21 days after the second application and 1, 3, 7, 14, 29, 61, 90, and 223 days after the third. Soil cores were divided and sampled as before.

Soil temperatures were 8.9-32.2°C at 15 cm and 7.2-26.7°C at 90 cm. Total irrigation was 11 cm and total rainfall 79.7 cm. The 0-15 cm soil layer was loam, pH 5.2, om 2.3%, clay 18%, CEC 61 mmol/kg.

Analysis indicated that the spray contained only 15% of the expected concentration (0.015 kg ai/hl), but the results were not accurate as the extraction efficiency of the analytical method was only 10%. The average amounts of formulation reaching the ground were calculated to be 1.7% ± 1.0% of the intended 0.28 kg ai/ha.

Soil samples were stored at –20°C for 3-11 months and analysed as before, but residues were only shown to be stable for 6 months (Table 107). All results were corrected for moisture content, concurrent method recoveries (70%-127%), and average field spike recoveries (68% for 0.5 mg/kg diflubenzuron, 75% for 0.1 mg/kg CPU, and 104% for 0.1 mg/kg DFBA), but not for interferences (<0.01 mg/kg).

Pre-treatment soil samples showed no residues of any of the test substances. Diflubenzuron was found only in the 0-15 cm soil layer. CPU was found for the first time on day 8 after the second application in the 0-15 cm layer and was below the LOQ from day 14 after the third application. DFBA was not found at all. The results for the 0-15 cm layer are summarized in Table 30. The author calculated a half-life of 6.6 days for diflubenzuron using a linear model with the logarithm of the concentration.

Other pesticides were applied during the study, none of which are structural analogues of diflubenzuron. A correct half-life for diflubenzuron and CPU could not be calculated, because the levels found 3 days after the third application were below the LOQ. The model used by the author to calculate the half-life of diflubenzuron is unclear. The actual application rate was very low at 1.7% of the intended dose. The half-life is not considered reliable.

Table 30. Soil residues, average concentrations in the 0-15 cm layer on a dry weight basis.

Application	Days after treatment	Diflubenzuron (mg/kg)	CPU (mg/kg)	DFBA (mg/kg)
1st spray	0	0.039	<0.01	<0.01
	1	0.022	<0.01	<0.01
	3	<0.01	<0.01	<0.01
	7	<0.01	<0.01	<0.01
	14	<0.01	<0.01	<0.01
2nd spray	0	0.050	<0.01	<0.01
	1	0.086	<0.01	<0.01
	3	0.028	<0.01	<0.01
	8	0.045	0.023	<0.01
	14	0.024	0.016	<0.01
3rd spray	0	0.037	0.015	<0.01
	1	0.033	0.017	<0.01
	3	<0.01	<0.01	<0.01
	7	0.038	0.020	<0.01
	14	<0.01	<0.01	<0.01
	29	<0.01	<0.01	<0.01
	61	<0.01	<0.01	<0.01
	89	<0.01	<0.01	<0.01

Another study was carried out with the same formulation on a plot of Melrose apple trees in Woodburn, Oregon, USA, according to the same EPA guideline (Ver Hey, 1991b,c, GLP). Three applications at 0.28 kg ai/ha per application were made at intervals of 21 days in June-July, 1989. The treated plot (0.41 ha) consisted of five sub-plots. Soil samples were collected before the first application, on days 0, 1, 3, 7 and 14 after each application and additionally on days 30, 60, 90, 180, 270 and 360 after the third application. Soil cores were divided and sampled as before.

Soil temperatures ranged from 11.7 to 20.6°C at 15 cm and from 8.3 to 18.9°C at 90 cm. Total irrigation was 5096 mm and the total rainfall 1325 mm, an increase in total water supply of 25% compared to the two wettest years in the preceding ten-year period. The 0-15 cm soil layer was silt loam, pH 5.5, om 3.6%, clay 20%, CEC 86 mmol/kg.

Analysis indicated that the sprays contained 73%, 96% and 113% of the expected concentration (0.0096 kg ai/hl) for the first, second and third application respectively, and that the average amounts of formulation reaching the ground were 38%, 25% and 33% of the amounts applied (0.28 kg ai/ha).

Soil samples were stored at –20°C for 7 days to 8 months and stability for 456 days was demonstrated (Table 111). Residues were determined as before. Results were corrected for moisture content and concurrent method recoveries (64%-136%). but not for average field spike recoveries (101% for 0.5 mg/kg diflubenzuron, 78% for 0.1 mg/kg CPU and 109% for 0.1 mg/kg DFBA) or matrix interferences (max 0.03 mg/kg for diflubenzuron, 0.01 mg/kg for CPU, 0.02 mg/kg for DFBA).

Pre-treatment soil samples showed no residues of any of the test substances, and an irrigation water sample contained no diflubenzuron. The results of soil analyses of the 0-15 cm layer are summarized in Table 31. Diflubenzuron was found in all but one of the 0-15 cm samples and at 0.01-0.03 mg/kg in 3 samplings in the 15-30 cm soil layers (14 days after the second application, and 7 and 30 days after the third). No residues above the LOQ (0.01 mg/kg) were found in the deeper layers. CPU was found for the first time on day 7 after the first application in the 0-15 cm layer and was below the LOQ on day 360 after the third application. DFBA was found for the first time at the LOQ on day 7, after the first application and was at or below the LOQ thereafter. The CPU results are not considered valid because of low precision, and results below 0.1 mg/kg diflubenzuron and 0.07 mg/kg DFBA are not considered valid because of matrix interferences.

The author calculated a half-life of 68 days for diflubenzuron, using linear regression with a log-transformed first order exponential equation, based on the residues on days 0 to 180 after the third application.

The half-life recalculated by linear regression of log concentration against time ($r^2 = 0.69$) was 68 days, as calculated by the author. The value is not considered reliable because of matrix interferences in the control soil.

Table 31. Soil residues, average concentrations in the 0-15 cm layer on a dry weight basis.

Application	Days after treatment	Diflubenzuron (mg/kg)	CPU (mg/kg)	DFBA (mg/kg)
1st spray	0	0.08	<0.01	<0.01
	1	0.05	<0.01	<0.01
	3	0.05	<0.01	<0.01
	7	0.06	0.01	0.01
	14	0.03	0.01	<0.01
2nd spray	0	0.06	<0.01	0.01
	1	0.11	0.01	<0.01
	3	0.08	0.01	<0.01
	7	0.09	0.03	<0.01
	14	0.01	0.02	<0.01
3rd spray	0	0.12	0.02	0.01
	1	0.11	0.02	<0.01
	3	0.08	0.02	0.01
	7	0.06	0.02	0.01
	14	0.07	0.02	0.01
	30	0.07	0.04	<0.01
	60	0.02	0.03	<0.01
	90	0.02	0.04	<0.01
	180	0.02	0.02	0.01
	270	<0.01	0.02	<0.01
	360	0.01	<0.01	<0.01

In a study with the WP 250 formulation on bare soil in West Memphis, Arkansas, USA, according to the EPA guideline (Kramer, 1992, GLP) three applications of 0.14 kg ai/ha 7 days apart were made to five replicate field plots, each 66 m^2, in June 1990. Soil samples were collected one day before the first application and on days 0, 1, and 3 after each application and 7, 14, 30, 60, 90, 180, 270, 360, 450, and 540 days after the third application. Soil cores were divided and triplicate composite samples prepared as before.

The soil temperature ranged from 3.3 to 31.7°C at 15 cm and from 12.2 to 28.9°C at 90 cm. The total irrigation was 1730 mm and the total rainfall 1141 mm. The 0-15 cm soil layer was silt loam, pH 6.9, om 2.0%, clay 15%, CEC 77 mmol/kg.

Analysis indicated that the spray contained 87%, 76% and 79% of the expected concentration (0.050 kg ai/hl) for the first, second and third applications respectively, and that the average amounts of formulation reaching the ground were 68%, 92% and 79% of the amount applied (0.14 kg ai/ha).

Soil samples were stored at –20°C for 1-11 months and residues were shown to be stable for 400 days (Table 109). Residues were determined as before. Results were corrected for moisture content and concurrent method recoveries (70%-128%), but not for average field spike recoveries (106-157% for 0.5 mg/kg diflubenzuron, 94-118% for 0.1 mg/kg CPU, 86-101% for 0.1 mg/kg DFBA) or interferences (<0.01 mg/kg).

Pre-treatment soil samples showed no residues of any of the test substances and an irrigation water sample showed no diflubenzuron. The results for the 0-15 cm layer are summarized in Table 32. In the upper 15 cm layer, diflubenzuron was below the LOQ of 0.01 mg/kg on day 60 after the third application, and CPU was found on day 3 after the first application at 0.011 mg/kg and in concentrations of about 0.01 mg/kg after the third application. No residues were found in the other soil layers and DFBA was not found at all.

The reviewer recalculated the half-life of diflubenzuron after the third application to be 11 days (r^2=0.62), as reported by the author. It was not possible to calculate half-lives for DFBA or CPU.

Other pesticides were applied during the study, none of which are structural analogues of diflubenzuron.

Table 32. Soil residues, average concentrations in the 0-15 cm layer on a dry weight basis.

Application	Days after treatment	Diflubenzuron (mg/kg)	CPU (mg/kg)	DFBA(mg/kg)
1st spray	0	0.049	<0.01	<0.01
	1	0.015	<0.01	<0.01
	3	0.020	0.011	<0.01
2nd spray	0	0.058	<0.01	<0.01
	1	0.032	0.011	<0.01
	3	0.015	<0.01	<0.01
3rd spray	0	0.051	0.013	<0.01
	1	0.025	0.010	<0.01
	3	0.021	0.012	<0.01
	7	0.021	<0.01	<0.01
	14	0.015	<0.01	<0.01
	30	lost	lost	lost
	60	<0.01	<0.01	<0.01
	90	<0.01	<0.01	<0.01

Photodegradation

The photodegradation of diflubenzuron was determined in the laboratory (Daun, 1988, GLP). Air dried, 2 mm-sieved soil in petri dishes was mixed with a small amount of water to a uniform slurry and air-dried. 500 µl of an aqueous suspension of [^{14}C]diflubenzuron (labelled in both rings, radiochemical purity 97.9%, specific activity 14.7 mCi/g) was evenly distributed onto the surface of each soil sample at a concentration of 5 mg/kg soil. Samples were incubated in a xenon lamp chamber at 24 to 29°C in a cooling tray covered with a pyrex glass plate and sealed with teflon tape. Dark controls were incubated at 25 ± 1°C. Volatiles were trapped in ethylene glycol (trap 1) and 2-ethoxyethanol:etholamine (1:1; trap 2). Duplicate plates of irradiated soil were sampled after 0, 1, 2, 4, 8, 20 and 33 days. Dark controls were sampled after 1, 2, 4, 9, 21 and 35 days. Analysis was by LSC and HPLC-UV after extraction with ACN and methanol. Soil-bound radioactivity was determined by LSC after combustion.

Irradiation was interrupted by power failures for 35 hours between days 5 and 7 and for 4 hours on day 27. Periods of irradiation in the following text are corrected for this.

The mean recovery of radioactivity was between 86% and 100% of that applied.

In irradiated samples extractable radioactivity decreased from 96% of that applied on day 0 to 68% on day 33, and diflubenzuron from 99% on day 0 to 61% on day 33. Early-eluting HPLC fractions, encompassing CPU and DFBA, amounted to a maximum of 4.3% on day 33. Soil-bound radioactivity after initial extraction increased from 2.1% of the applied radioactivity on day 0 to 18% on day 33. The half-life was calculated as 68 days by linear regression.

In the dark controls the extractable radioactivity decreased from 96% on day 0 to 87% on day 35, diflubenzuron from 96% on day 0 to 84% on day 35, and DFBA was found at 0.1-2.1%, the maximum on day 2. Early-eluting HPLC fractions amounted to a maximum of 2.3% of the applied radioactivity on day 35. Soil-bound radioactivity increased from 2.1% of that applied on day 0 to 14% on day 35. No half-life was calculated.

The half-life of 68 days with continuous irradiation equals 82 12-hour days of natural sunlight at 40° N latitude. The results are considered reliable.

In another laboratory experiment (Saxena *et al.*, 1992, GLP) an aqueous suspension of [^{14}C]diflubenzuron (labelled in both rings, chemical purity >99.5%, radiochemical purity >99%, specific activity 9.91 mCi/g) was evenly distributed onto the surface of air-dried, 2 mm-sieved soil on stainless steel test plates (5 cm diameter, 2 mm thick) and the soils brought to about 75% of the water holding capacity. The plates were incubated at 25 ± 1°C in an airtight vessel covered with a quartz glass plate and with a heat exchanger in the bottom. Dark controls were incubated at 25 ± 1°C. Volatiles were trapped in NaOH. Duplicate plates of irradiated and control soils were sampled after 0, 1, 2, 4, 7, 10 and 16 days. Analysis was by LSC and HPLC-UV after extraction with ACN and methanol. Soil-bound radioactivity was determined by LSC after combustion, and this fraction was analysed further after extraction with ACN and phosphoric acid at room temperature and under reflux. Identification was by 2D-TLC. Microbial activity was determined before dosing and after 7 and 16 days.

The mean recovery of radioactivity was between 99% and 103% of that applied. Microbial activity before the experiment was 4.19 x 10^6 CFU/g dw soil.

In irradiated samples microbial activity was 3.45 x 10^7 CFU/g dw soil after 7 days and 1.05 x 10^8 CFU/g after 16 days. Extractable radioactivity decreased from 100% on day 0 to 55% on day 16 and diflubenzuron from 99% on day 0 to 40% on day 16. DFBA was found at 1.4 to 3%, the maximum on day 7. CPU reached a maximum of 13% on day 10 and 11% was found on day 16. Two unknown compounds were found in amounts up to 0.6% of the applied radioactivity. Volatiles increased to 9% of the applied radioactivity on day 16, and soil-bound radioactivity after initial extraction from 0.7% to 35%. Diflubenzuron in the acid-extracted soil-bound fraction accounted for 4.5% of the applied radioactivity, and DFBA and CPU together for 19%. The half-life was calculated as 11 days by linear regression.

In the controls microbial activity was 2.43 x 10^7 CFU/g dw soil after 7 days and 9.88 x 10^6 CFU/g after 16 days. Extractable radioactivity decreased from 93% on day 0 to 15% on day 16 and diflubenzuron from 86% to 6.2%. DFBA was found at 0.1% to 2.1%, the maximum on day 2. CPU reached a maximum of 21% on day 7 and was 6.7% on day 16. Two unknown compounds were found in amounts up to 3.5% of the applied radioactivity. Volatiles increased to 23% of the applied radioactivity on day 16, and soil-bound radioactivity after initial extraction from 7.3% to 58%. Diflubenzuron in the acid-extracted soil-bound fraction accounted for 1.7% of the applied radioactivity, and DFBA and CPU together for 19%. The half-life was calculated as 3.7 days.

Degradation in irradiated samples was slower than in dark controls. Microbial activity in the soils increased during the test period and degradation is likely to be biodegradation rather than photodegradation. The results are not considered reliable.

Adsorption/desorption

The adsorption and desorption of diflubenzuron (purity >95%) was determined in four soils and four sediments (Booth *et al.*, 1981, non-GLP). Diflubenzuron was added to 25g of each soil or sediment with 50 ml of distilled water to obtain concentrations of 0.5 and 2 mg/l in 200 ml Erlenmeyer flasks which were shaken at 300 rpm for 10 min. Samples of the soil/water mixture were taken after 5, 10, 20 and 60 min and centrifuged at 2000 rpm for 5 min. 1 ml of each supernatant was analysed by LSC. For the desorption experiment, the soil/water mixture was filtered through a Büchner funnel and the filtered soil was shaken for 60 min with 50 ml of distilled water. Samples were taken after 5, 10, 20 and 60 min and concentrations in the water were determined by LSC after centrifugation.

After 5 min, 1.5 to 8.2% of the applied radioactivity was in the water phase. Equilibrium was approached within the next 55 min except in silt loam soil, where concentrations in water tended to increase after the initial sorption.

Comments. Concentrations were expressed as cpm/ml, cpm/25 g soil and percentages of the applied radioactivity. The test concentrations were above the solubility of diflubenzuron in water (0.2 mg/l). The soil:water ratio was too low. Only the water phase was analysed. For both test concentrations, sorption coefficients were recalculated as the quotient of cpm/g soil and cpm/ml water using the raw data for the 60 min samples.

The relatively low sorption by silt loam soil cannot be explained by its clay or silt content. There is no indication of concentration dependence. The $K_{s/l}$s and K_{om}s in Table 33 are averages of the experiments with 0.5 and 2 mg diflubenzuron/l. All the recalculated K_{om}s in Table 33 are considered reliable.

Table 33. Sorption and partition coefficients of diflubenzuron in four soils and four sediments.

Soil or sediment	Om (%)	pH	CEC (mmol/kg)	Clay (%)	$K_{s/l}$ (l/kg)	K_{om} (l/kg)
Soils						
loamy sand	0.56	6.1	57.0	3	33	5822
clay	2.6	5.8	418	66	144	5628
silt loam	0.81	8.4	49.4	10.8	16	1920
sandy clay loam	2.0	5.5	97.3	24.8	165	8462
Sediments						
sandy loam	1.3	8.1	287	20	168	12727
silty clay loam	4.8	8.5	387	42	181	3761
sandy loam	4.2	8.1	297	12	84	2012
sandy clay loam	0.97	7.8	248	22	75	7768

The adsorption of DFBA (purity 96.4%) was determined in sand, sandy loam, and loam soils according to the OECD 106 guideline (Van Dijk, 1992b, non-GLP). Soils were 2 mm-sieved and portions of 2 g were shaken for 16 hours with 10 ml of a solution of DFBA in 0.01 M $CaCl_2$ (concentration about 5 mg/l). Tubes without soil, tubes with soil, and a $CaCl_2$ solution were used as controls. Tests were run in duplicate. Suspensions were centrifuged and supernatants analysed for

DFBA. Desorption was determined by adding 10 ml 0.01 M $CaCl_2$ to the solid residue, shaking for 16 hours and analysing the aqueous phase after centrifugation. Analyses were by ion suppression chromatography with UV detection.

Concentrations of DFBA in the solutions were 4.8-5.0 mg/l, corresponding to 1.4-2.4% sorption. There were no significant differences in sorption between the soils. After desorption, the solutions contained 0.32-0.47 mg/l DFBA.

Only one concentration was tested, and only the water phase was analysed. Sorption was too low to calculate K_{om}.

Table 34. Properties of the three soils used in the adsorption experiment (Van Dijk, 1992b).

Soil	om (%)	pH	CEC (mmol/kg)	Clay (%)
sand	7.4	4.6	106	2.7
sandy loam	2.8	5.7	65	5.2
loam	4.5	7.3	159	16.5

The adsorption of ring-labelled [^{14}C]CPU (chemical purity 99.2%, radiochemical purity 99.0%, specific activity 11.0 mCi/g) was determined in four soils according to OECD 106 and EPA 163-1 guidelines (Van Dijk, 1992a, 1999, GLP). The equilibration time was determined by shaking 2 g portions of soil with 10 ml of a 20 mg/l solution of CPU in 0.01 M $CaCl_2$ for 0-16 hours. For the adsorption experiment, 2 g portions of soil were shaken with 10 ml of the CPU solution for 16 hours and analysing the aqueous phase after centrifugation. Organic extracts of soil were analysed for radioactivity, and after organic extraction the remaining soil by combustion and LSC if the total recovery by organic extraction was <95%.

The total recovery of radioactivity after organic extraction was >95% from sand 1, sandy loam and loam and 88%-99% from sand 2. The recovery from sand 2 was 101%-105% after additional combustion. The average recovery was 98%-103%. Freundlich K_F and 1/n values were estimated by linear regression of log-transformed concentrations in water versus concentrations in soil (Table 35).

Comments. Equilibrium concentrations in soil were calculated from concentrations in the water phase. Freundlich exponents 1/n were below 1. The K_{om} values of 171, 140, 144, and 123 l/kg are considered reliable.

Table 35 Adsorption of CPU by four soils.

Soil	T (°C)	om (%)	pH	CEC (mmol/kg)	Clay (%)	K_F (l/kg)	(1/n)	K_{om} (l/kg)
sand 1	21	0.7	6.0	49	3.5	1.2	0.74	171
sand 2	21	4.3	5.5	106	2.7	6.0	0.79	140
sandy loam	21	1.6	6.6	65	5.2	2.3	0.79	144
loam	21	2.6	7.5	159	16.5	3.2	0.79	123

Leaching

Two soils were treated with ring-labelled [^{14}C]CPU (chemical purity 98.9%, radiochemical purity 96%, specific activity 5.4 mCi/g) at a rate equivalent to 0.12 kg/ha (Van Dijk and Groeneveld, 1986, non-GLP). In the report the soils are referred to as humic sand (for sand) and sandy clay (for loam). A column (7 cm diameter) was prepared with 20 cm coarse sand, 30 cm of untreated soil, about 1 cm treated soil, 2 cm untreated soil and 2 cm coarse sand. After saturation the column was leached with 2 l deionized water. The leachate was collected in fractions and the ^{14}C content determined by LSC. The column was sliced and radioactivity in the soil segments was determined by combustion analysis. The reported LOQ was 0.024 mg/kg dry soil. Recoveries of CPU from the soil columns after leaching are shown in Table 36.

Comments. The penetration depths were 0.5 cm in sand and 3.5 cm in loam. The calculated $K_{s/l}$ were 71.2 l/kg for sand and 9.93 l/kg for loam, and the K_{om} values 1548 and 276 l/kg respectively. The results are considered reliable.

Table 36 Recovery of CPU from soil columns after leaching.

	^{14}C, % of applied	
	sand	loam
total recovery	74-89	49-94
treated soil layer	64-70	11-14
untreated soil layer 0-2 cm	10-19	7-22
untreated soil layer 2-4 cm	nq	19-31
untreated soil layer 4-6 cm	nq	12-0
untreated soil layer 6-8 cm	nq	nq-20
untreated soil layer 8-10 cm	nq	nq-7
untreated soil layer 10-30 cm	nq	nq

nq: below LOQ (<0.05%)

Two reviews of the mobility studies in soil were submitted which contain no original data (Nimmo, 1985, Nimmo and Raven, 1986). They are identical except for the addition of a summary of a computer modelling study in the review by Nimmo and Raven.

Rotational crop studies

In a confined rotational crop study on soya beans, maize and potato in a greenhouse, sandy loam soil (pH 6.8; 2.4% om; 14% clay (<2 µm); CEC 14.3 mequivalents/100g) in buckets was treated with a suspension of diflubenzuron in which the difluorobenzoyl moiety was labelled with ^{3}H (specific activity 22.9 mCi/mmol; radiochemical purity 99.0%) and the chloroaniline moiety with ^{14}C (specific activity 2.35 mCi/mmol; radiochemical purity 99.4%). The dosage rate was reported only as 1.8 mg ai/bucket. After an ageing period of 10 weeks, soya bean seedlings (Glycine Max), maize seedlings (Caldera) and potato tubers (Libertas) were planted and grown in the buckets. Soil samples were collected 0, 2, 8, 15 and 24 weeks after treatment, leaf plus stem samples 15 and 19 weeks after treatment, and leaves at harvest. Soya beans, maize cobs and potato tubers were collected at harvest 23, 26 and 22 weeks after treatment respectively. Storage conditions were not reported (Nimmo and De Wilde, 1976b, non-GLP).

Homogenized plant and soil samples were extracted successively with ACN and boiling methanol/water (1:1). Extracts and unextractable residues were subjected to combustion and LSC. Diflubenzuron was determined by reversed isotope dilution analysis. Plant extracts were characterized by TLC (silica gel; 2 solvent systems) followed by autoradiography. The reference compounds were diflubenzuron and CPU.

Of the total applied radioactivity 61%-78% of the ^{14}C and 10%-50% of the ^{3}H was recovered from the soil 2-24 weeks after application; the recovery from the plants was not reported.

In the soil the extractable residues decreased from 95% to 10% of the total ^{14}C and 95% to 27% of the ^{3}H, and the bound residues increased (Table 37). In the period before planting (0-8 weeks after treatment) the [^{14}C]diflubenzuron decreased from 95% of the TRR (0.9 mg/kg) to 4.5% (<0.01 mg/kg) in the soil.

In the period before harvest (15-19 weeks after treatment), the extractable ^{14}C and ^{3}H residues in leaves were 0.02-0.18 mg/kg as diflubenzuron and the bound residues ≤0.01 mg/kg. At harvest, 22-26 weeks after treatment, the total extractable residues in the leaves, soya bean seeds, maize cobs and potato tubers were <0.05 mg/kg (^{14}C) and <0.02 mg/kg (^{3}H). At harvest the total bound residues in soya bean leaves and seeds were 0.06-0.07 mg/kg (^{14}C) and 0.01-0.02 mg/kg (^{3}H), and in maize leaves and cobs and potato leaves and tubers <0.005 mg/kg (Table 38). In the plant extracts, traces of CPU, possibly diflubenzuron and 2 unidentified metabolites were found.

Table 37. Distribution of radioactivity in soil (confined rotational crop study).

WAT	^{14}C						3H			
	TRR		extracted	diflubenzuron	unknown	bound	TRR		extracted	bound
	% of applied	mg/kg	% of TRR	% of TRR	% of TRR	% of TRR	% of applied	mg/kg	% of TRR	% of TRR
0	106	0.90	95	95	0.0	5.3	106	0.90	95	5.3
2	78	0.80	79	14	64	21	50	0.45	89	11
8	61	0.60	64	4.5	59	36	50	0.45	89	11
15	78	0.11	33	3.6	30	67	33	0.05	67	33
24	74	0.11	10	0.0	10	90	10	0.01	27	73

WAT: weeks after treatment
TRR: total recovered radioactivity;
Unknown: extracted residues minus diflubenzuron

Note. Because a separate bucket was used for each period after treatments, identical "% of applied" values do not correspond to identical "mg/kg" values.

Table 38 Distribution of radioactivity in the rotational crops (soya bean, maize and potato).

crop	sample	WAT	^{14}C, mg/kg as diflubenzuron			^{3}H, mg/kg as diflubenzuron		
			extracted	bound	TRR	extracted	bound	TRR
soya bean	aerial parts	15	0.15	<0.01	0.15	0.15	0.01	0.16
	aerial parts	19	0.07	<0.01	0.07	0.04	<0.01	0.04
	leaves	23	<0.05	0.06	0.06	<0.02	0.01	0.01
	seeds	23	<0.05	0.07	0.07	<0.02	0.02	0.02
maize	aerial parts	15	0.09	<0.005	0.09	0.02	<0.005	0.02
	aerial parts	19	0.08	<0.005	0.08	0.06	<0.005	0.06
	leaves	26	<0.05	<0.005	<0.06	<0.02	<0.005	<0.02
	cobs	26	<0.05	<0.005	<0.06	<0.02	<0.005	<0.02
potato	aerial parts	15	0.05	<0.005	0.05	0.18	<0.005	0.18
	aerial parts	19	0.09	<0.005	0.09	0.14	<0.005	0.14
	leaves	22	<0.02	<0.005	<0.02	<0.02	<0.005	<0.02
	tubers	22	<0.005	<0.005	<0.01	<0.005	<0.005	<0.01

WAT: weeks after treatment
TRR: total recovered radioactivity

In a field metabolism study on wheat, cabbage and onions the bare soil of a field plot in the USA (Greenville, Mississippi) was sprayed twice with diflubenzuron (^{14}C label in both rings; specific activity 17.8 mCi/mg; radiochemical purity 99%) at 0.067 kg ai/ha (WP formulation; 600 l water/ha) with an interval of 15 days. Wheat, onions and cabbages (varieties not reported) were planted 2 months after the last treatment and collected 3.5 months later. Soil was removed from the cabbage and onion samples. Plant samples were stored at –10°C until analysis (storage time not reported). Homogenized plant samples were subjected to combustion and LSC (Danhaus and Sieck, 1976, non-GLP).

Radioactivity in plant samples was below 0.01 mg/kg diflubenzuron equivalents. Soil residues were not measured.

In another field metabolism study diflubenzuron (^{14}C-labelled in both rings; specific activity 17.4 mCi/mmol; radiochemical purity 99.0%) was sprayed onto field-grown cotton (Stoneville 213) six or ten times at 5-day intervals (WP 250; 0.07 kg ai/ha in 93.5 l/ha water and 9.35 l/ha emulsified crop oil for each application; sandy loam soil). After harvest of the cotton, 90% of the cotton plant material was distributed over the surface of the treated plots and cultivated into the top 10 cm of soil. Wheat seed and collard seedlings were planted after 3 weeks, and radish and pinto bean seeds after 6 months (Bull and Ivie, 1978, non-GLP).

Soil, rotational crops, solvent extracts and remaining solids were subjected to combustion and LSC. Soil was extracted successively with ethyl acetate, acetone, methanol and acetone/water (19+1). Soil extracts were characterized by 2D-TLC (silica gel) and isolated compounds were identified by direct insertion probe MS.

Radioactive residues were generally low in the rotational crops, especially in the edible portions. After 6 sprays residues were <0.01-0.05 mg/kg dw diflubenzuron equivalents, except in native grass and weeds where they were 0.06-0.11 mg/kg (DAT 195). After 10 sprays residues were slightly higher at <0.01-0.05 mg/kg dw diflubenzuron equivalents in green pinto bean pods (DAT 237), mature pinto bean seeds (DAT 262) and mature wheat heads and plants (DAT 255), 0.06-0.10 mg/kg in collards (DAT 178), immature pinto bean plants (DAT 213), mature radish tops and tubers (DAT 255), and 0.10-0.20 mg/kg in native grass (DAT 178), green pinto bean plants (DAT 237) and mature pinto bean husks and plants (DAT 262).

Post-harvest residues of diflubenzuron in soil were located in the top 10 cm at levels of 0.10 mg/kg diflubenzuron equivalents after 63 days and 0.30 mg/kg after 46 days from 6 and 10 sprays respectively when 90% of the treated plant materials were incorporated into the soil. Diflubenzuron residues were persistent during the subsequent winter and spring months (0.04-0.05 mg/kg dw diflubenzuron equivalents from 6 applications and 0.29-0.30 mg/kg dw from 10 applications), but declined slightly with the onset of high summer temperatures (0.02-0.04 mg/kg from 6 applications and 0.10-0.13 mg/kg from 10). Soil collected in the spring (DAT 215; 0.30 mg/kg dw diflubenzuron equivalents, 10 applications) revealed that 87% of the TRR was extractable and contained diflubenzuron (81% of the TRR), CPU (1.7%) and two unknowns (each <2%). In soil collected in the following autumn (DAT 373; 0.10 mg/kg dw, diflubenzuron equivalents, 10 applications) 79% of the TRR was extractable and was all identified as diflubenzuron.

In rotational crop residue trials Gaydosh (1999d, GLP) treated cotton with 6 applications of a WP 250 formulation, the first three before and the last three after boll opening (each 0.28 kg ai/ha; total 1.7 kg ai/ha). The last application was 14 days before harvest. The spray volume was 112-140 l/ha for each application (total 0.20-0.25 kg ai/hl). Applications were by tractor-operated boom sprayer. After harvest each plot (669-697 m^2) was divided into three equal sub-plots, one for each rotational crop (lettuce, turnips and wheat). Rotational crops were planted 30, 45, 60, 90, 120, 180 and 365 days after the last treatment and collected from 12 areas (duplicate plots, 2 field samples/plot): wheat forage (1.1 kg) 6-8 weeks after planting, wheat hay (0.7 kg) at early milk or dough stage, and wheat grain (1.4 kg), wheat straw (0.9 kg), turnip tops (1.8 kg), turnip roots (1.8 kg) and lettuce (1.8 kg) at maturity. No unusual weather conditions were experienced (1995-1997, temperature -14-45°C; total rain 65-236 cm) except in East Bernard, where October 1995-May 1996 was abnormally dry and August-September 1996 and January-April 1997 were abnormally wet. Several samples could not be collected owing to the weather conditions, and some wheat samples were destroyed by birds.

The total storage time (<0°C) from harvest to last analysis was 77-513 days for diflubenzuron, 54-407 days for CPU, and 184-684 days for PCA. The samples were analysed in duplicate by separate methods for each analyte: GC-ECD method LAI 3-86-6 for diflubenzuron, GC-ECD method LAI-3-86-9 for CPU and GC-ID-MS method I for PCA. Concurrent method recoveries and interferences were reported, but results (Table 39) were not corrected for them.

Table 39. Residues of diflubenzuron, CPU and PCA in rotational crops planted after cotton which was treated with 6 applications of diflubenzuron at 0.28 kg ai/ha.

Sample	Plantback interval[1] (days)	PHI[2] (days)	diflubenzuron (mg/kg)	CPU (mg/kg)	PCA (mg/kg)
USA, Madera (CA)[3]					
wheat forage	30	53	<0.01 (3); 0.01	0.01 (4)	<0.005 (4)
wheat straw	30	200	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat grain	30	200	<0.01 (4)	<0.01 (3); 0.02	<0.005 (4)
wheat hay	30	150	<0.01 (4)	<0.01 (4)	<0.005 (4)
lettuce	30	154	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip tops	30	95	<0.01 (4)	0.01 (2); 0.02 (2)	<0.005 (4)
turnip root	30	95	<0.01 (4)	<0.01 (2); 0.01; 0.04	<0.005 (4)
wheat forage	45	56	<0.01 (4)	<0.01; 0.01 (2); 0.02	<0.005 (4)
wheat straw	45	193	<0.01 (3); 0.01	<0.01 (3); 0.02	<0.005 (4)
wheat grain	45	193	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat hay	45	139	<0.01 (4)	<0.01 (4)	<0.005 (4)
lettuce	45	184	<0.01 (2); 0.01; 0.02	<0.01 (4)	<0.005 (4)
turnip tops	45	93	<0.01 (4)	0.01 (4)	<0.005 (4)
turnip root	45	93	<0.01 (4)	0.01 (3); 0.02	<0.005 (4)
wheat forage	61	53	<0.01 (4)	<0.01 (3); 0.01	<0.005 (4)
wheat straw	61	177	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat grain	61	177	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat hay	61	151	<0.01 (4)	<0.01 (4)	<0.005 (4)
lettuce	61	168	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip tops	61	99	<0.01 (4)	0.02 (4)	<0.005 (4)
turnip root	61	99	<0.01 (4)	0.02 (4)	<0.005 (4)
wheat forage	92	62	<0.01 (1); 0.01 (3)	<0.01 (2); 0.01; 0.02	<0.005 (4)
wheat straw	92	154	<0.01; 0.01 (3)	<0.01 (4)	<0.005 (4)
wheat grain	92	154	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat hay	92	131	<0.01 (4)	<0.01 (4)	<0.005 (4)
lettuce	92	146	<0.01 (4)	<0.01 (2); 0.01; 0.02	<0.005 (4)
turnip tops	92	92	<0.01 (4)	0.01 (4)	<0.005 (4)
turnip root	92	92	<0.01 (4)	<0.01 (2); 0.02 (2)	<0.005 (4)
wheat forage	120	57	<0.01 (2); 0.01 (2)	<0.01 (4)	<0.005 (4)
wheat straw	120	162	<0.01 (3); 0.01	<0.01 (4)	<0.005 (4)
wheat grain	120	162	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat hay	120	109	<0.01 (4)	<0.01 (4)	<0.005 (4)
lettuce	120	131	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip tops	120	103	<0.01 (4)	0.03 (4)	<0.005 (4)
turnip root	120	103	<0.01 (4)	0.01 (3); 0.02	<0.005 (4)
wheat forage	180	49	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat straw	180	128	<0.01 (3); 0.01	<0.01 (4)	<0.005 (4)
wheat grain	180	128	<0.01 (3); 0.01	<0.01 (4)	<0.005 (4)
wheat hay	180	78	<0.01 (4)	<0.01 (4)	<0.005 (4)
lettuce	180	86	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip tops	180	66	<0.01 (4)	0.02 (2); 0.03 (2)	<0.005 (4)
turnip root	180	66	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat forage	365	55	<0.01 (3); 0.01	<0.01 (4)	<0.005 (4)
wheat straw	365	232	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat grain	365	-	na	na	na
wheat hay	365	152	<0.01 (4)	<0.01 (4)	<0.005 (4)
lettuce	365	113	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip tops	365	62	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip root	365	62	<0.01 (4)	<0.01 (4)	<0.005 (4)
USA, East Bernard, TX[4]					
wheat forage	33	-	na	na	na
wheat straw	33	260	0.01 (4)	<0.01 (4)	<0.005 (4)
wheat grain	33	260	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat hay	33	208	<0.01 (4)	<0.01 (4)	<0.005 (4)
lettuce	31	116	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip tops	31	93	<0.01 (4)	<0.01 (4)	<0.005 (4)

Sample	Plantback interval[1] (days)	PHI[2] (days)	diflubenzuron (mg/kg)	CPU (mg/kg)	PCA (mg/kg)
turnip root	31	93	<0.01 (4)	0.01 (2); 0.02; 0.06	<0.005 (4)
wheat forage	45	-	na	na	na
wheat straw	45	248	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat grain	45	248	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat hay	45	200	<0.01 (4)	<0.01 (4)	<0.005 (4)
lettuce	45	102	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip tops	45	102	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip root	45	102	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat forage	60	-	na	na	na
wheat straw	60	233	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat grain	60	233	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat hay	60	193	<0.01 (4)	<0.01 (4)	<0.005 (4)
lettuce	60	63	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip tops	60	63	<0.01 (4)	0.03 (4)	<0.005 (4)
turnip root	60	63	<0.01 (4)	<0.01 (3); 0.01	<0.005 (4)
wheat forage	94	-	na	na	na
wheat straw	94	-	na	na	na
wheat grain	94	-	na	na	na
wheat hay	94	-	na	na	na
lettuce	94	123	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip tops	94	115	<0.01 (4)	0.01 (2); 0.02 (2)	<0.005 (4)
turnip root	94	115	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat forage	119	-	na	na	na
wheat straw	119	-	na	na	na
wheat grain	119	-	na	na	na
wheat hay	119	-	na	na	na
lettuce	119	-	na	na	na
turnip tops	119	-	na	na	na
turnip root	119	-	na	na	na
wheat forage	180	55	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat straw	180	-	na	na	na
wheat grain	180	-	na	na	na
wheat hay	180	-	na	na	na
lettuce	180	82	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip tops	180	-	na	na	na
turnip root	180	-	na	na	na
wheat forage	365	55	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat straw	365	269	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat grain	365	269	<0.01 (4)	<0.01 (4)	<0.005 (4)
wheat hay	365	237	<0.01 (4)	<0.01 (4)	<0.005 (4)
lettuce	365	153	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip tops	365	121	<0.01 (4)	<0.01 (4)	<0.005 (4)
turnip root	365	121	<0.01 (4)	<0.01 (4)	<0.005 (4)

[1] Interval between last cotton treatment and planting of rotational crop

[2] Interval between planting and harvest of rotational crop

[3] USDA soil type sandy loam (pH 7.7, om 0.2%, CEC 10.8, silt 24%). Crop varieties: cotton Accala Maxxa, wheat Yecora Rojo, lettuce Great Lakes 6S4, turnips Royal Crown.

[4] USDA soil type (pH 6.6, 1.1% om, CEC 13.7, 17% silt). Crop varieties: cotton DP&L50, wheat Mit, lettuce Oakleaf, turnips Purple Top White Globe.

Environmental fate in water-sediment systems

The Meeting received information on the degradation of diflubenzuron in water by physicochemical means and its biodegradation in activated sludge, water-sediment systems and ditch and canal water.

Physicochemical degradation in water. The hydrolysis of diflubenzuron was determined in the laboratory (De Wilde, 1985, non-GLP). Solutions of ^{14}C- and ^{3}H-labelled diflubenzuron (chemical purity, radiochemical purity and specific activity not reported) were added to sterile buffers of pH 5, 7 and 9, at concentrations of 0.11 mg/l and 0.66 mg/l respectively, and to a sterile buffer of pH 12 with both compounds at 0.11 mg/l. Solutions incubated in the dark at 20°C were sampled after 0, 21, 42 and 63 days (pH 5, 7 and 9) or 0, 7, 14 and 28 days (pH 12). Analysis was by TLC and reverse isotope dilution.

In a second test according to BBA guidelines solutions of 0.075 to 0.085 mg/l unlabelled diflubenzuron in sterile buffers of pH 5, 7 and 9 were incubated at 22°C in the dark. Samples were taken at 0, 30, 60, 90 and 150 days (pH 9 up to 90 days) and analysed by HPLC.

At pH 5 89% and 77% of the added ^{14}C- and ^{3}H-labelled diflubenzuron remained after 63 days at 20°C and 98% of the unlabelled substance was recovered after 150 days at 20°C, and at pH 7 69% and 73% remained after 63 days, and 101% was recovered after 150 days. At pH 9 35% of both labels remained after 63 days and 25% of the unlabelled substance was recovered after 150 days. At pH 12 8% of the double-labelled diflubenzuron remained after 28 days at 20°C.

DFBA and CPU were identified as degradation products.

Analytical recoveries were not given. Half-lives at pH 9 and pH 12 were recalculated from the author's data by linear regression of log % against time. The results are considered reliable.

Table 40. Hydrolysis of diflubenzuron in buffer solutions.

Compound	Duration, days	°C	pH	Transformation (%)	Half-life, days
[^{14}C]diflubenzuron	63	20	5	11	>63
[^{3}H]diflubenzuron	63	20	5	23	>63
[^{14}C]diflubenzuron	63	20	7	31	>63
[^{3}H]diflubenzuron	63	20	7	27	>63
[^{14}C]diflubenzuron	63	20	9	65	42
[^{3}H]diflubenzuron	63	20	9	65	40
[^{14}C-^{3}H]diflubenzuron	28	20	12	82	7.5
unlabelled diflubenzuron	150	22	5	2	>150
unlabelled diflubenzuron	150	22	7	0	>150
unlabelled diflubenzuron	90	22	9	75	44

Photodegradation in water was determined in the laboratory according to Fifra guidelines (Boelhouwers *et al.,* 1988a, GLP). Four replicate solutions of phenyl-labelled [^{14}C]diflubenzuron (chemical purity >97.1%, radiochemical purity >99.2%, specific activity 544 MBq/g) in double-distilled phosphate buffer (pH 7) with 1% ACN as co-solvents at a concentration of 171 µg/l were irradiated with a 450 W Xenon arc (λ ≥290 nm) for 15 days, equivalent to 30 days of natural sunlight, with two dark controls, at 25 ± 0.1°C. Samples were taken at 0 hours and 1, 3, 7 and 15 days. Volatiles were trapped in KOH. Analysis was by LSC and HPLC-UV, with identification by MS.

The mean analytical recovery from fortified samples was 96% with a reported LOQ of 0.004 mg/l. The initial recovery of ^{14}C from the irradiated samples was 94%, 97% of which was from diflubenzuron. After 15 days the recovery was 85%, 78% from diflubenzuron, and sorption to the glass walls accounted for the remaining 15% which was also present as unchanged diflubenzuron. The total amount of diflubenzuron in the irradiated samples thus represented about 81% of the initial radioactivity. The dark controls contained 93% of the initial radioactivity after 15 days, 99% of which was due to diflubenzuron. Volatiles were not found. The authors calculated the half-life as 40 days assuming zero order kinetics.

After 15 days DFBA, DFBAM and CPU represented 4, 1 and 8% of the recovered radioactivity. PCA was not found; an unknown peak accounted for 6% of the recovered radioactivity.

On the basis of this study, Van der Voorden (1993) calculated a photolytic quantum yield of 4.7×10^{-5} mole/einstein.

Sorption seems to be complete after 7 days. The half-life based on the measured diflubenzuron in solution is 32 days, and this includes photodegradation and sorption to glass. The half-life corrected for sorption is 47 days.

[^{14}C]diflubenzuron (labelled in both rings, radiochemical purity >99%, specific activity 16.14 mCi/mmole) in buffer solutions or water at a final concentration of 1 g/l was incubated at 121°C for 24 hours and at 36°C for 56 days (Ivie *et al.*, 1980, non-GLP). Samples were acidified to pH 2 with HCl, extracted four times with ethyl acetate and counted by LSC. Samples containing >1% of the applied radioactivity were adjusted to pH 12 with NaOH, extracted again four times, dried over anhydrous sodium sulfate and analysed by TLC and LSC. Degradation products were further purified by TLC if necessary and identified by MS or GC-MS.

At 121°C the total recovery of radioactivity was >99%. After 24 hours, diflubenzuron accounted for 1 to 6% of the applied radioactivity at pH 10, 4% to 51% at pH 6 and 11% to 50% at pH 4. The degradation products found are shown in Table 41.

Table 41 Degradation products of diflubenzuron in buffer at 121°C.

Compound	^{14}C, % of initial		
	pH 4	pH 6	pH 10
CPU	0	0-<1	0-<1
DFBA	0	1-9	28-35
DFBAM	30-54	26-50	18-25
PCA	14-36	15-33	28-39
N,N'-bis(4-chlorophenyl)urea	0	0	1-3
1-(4-chlorophenyl)-5-fluoro-(1*H*,3*H*)-quinazolinedione	0	2-11	5-8
2-[(4-chlorophenyl)amino]-6-fluorobenzoic acid	0	0	1-3

At 36°C the total recovery of radioactivity was >99%. At pH 10 diflubenzuron decreased from 99% of the applied radioactivity initially to 1.7% after 14 days and was not found thereafter; the half-life was <3 days. At pH 6, diflubenzuron decreased to 1.2% at 56 days, and the half-life was about 7 days. At pH 4 no degradation occurred. The degradation products found at pH 6 and 10 are shown in Table 42.

Table 42. Degradation products of diflubenzuron in buffer at 36°C.

Compound	^{14}C, max. % of initial at time shown			
	pH 6	days	pH 10	days
CPU	49.2	56	47.1	28
DFBA	44.3	56	45.0	14
DFBAM	2.9	28	4.6	56
1-(4-chlorophenyl)-5-fluoro(1H,3H)-quinazolinedione	1.5	28-56	3.5	56

<u>Biodegradation in activated sludge</u>. The biodegradation of diflubenzuron (purity 99.5%) was investigated in a closed-bottle experiment according to the OECD 301D guideline (Thus and Van der Laan-Straathof, 1993c, GLP). The inoculum was taken from an activated sludge plant and preconditioned to reduce the endogenous respiration rate by aeration for one week. The test was in bottles designed for biochemical oxygen demand tests, as follows.

- bottles with medium, OECD mineral salt without ammonium chloride to prevent nitrification.

- bottles with medium and inoculum at a concentration of 2 mg dw/L.
- bottles with the positive reference substance sodium acetate.
- bottles for the negative control with inoculum and silica gel.
- test bottles with diflubenzuron (2 mg/l), dissolved in dichloromethane and coated on silica gel, to guarantee the availability of diflubenzuron to the micro-organisms. The solvent was evaporated afterwards.

Seven bottles for each test series were incubated in a dark climate-controlled room. The oxygen content was measured after 5, 15, and 28 days in two bottles of each test series, and the pH at 0, 5, and 28 days. The biodegradation was calculated from the ratio of the biochemical oxygen demand to theoretical oxygen demand.

The inoculum activity was good: more than 60% of the sodium acetate was degraded within 5 days and more than 70% within 15 days in the positive control. No degradation of diflubenzuron was found. The pH in the test bottles varied between 7.3 and 7.6.

Only three sample points were measured. There was no toxicity control (test substance + reference substance), although the authors reported that no inhibition of the oxygen consumption was found in the presence of sodium acetate and diflubenzuron in preliminary tests. The results of the preliminary tests were not reported. The test substance was coated onto silica gel, which could interfere with the bioavailability of the test substance for the micro-organisms. The test concentration was far above the water-solubility of diflubenzuron (0.2 mg/l at 20°C). The results are not considered reliable.

The biodegradation of [^{14}C]diflubenzuron (both phenyl rings labelled, radiochemical purity >99.4%, chemical purity >99.1%, specific activity 19.5 mCi/g) was investigated in a modified Sturm test according to the OECD 301B guideline by Van der Laan-Straathof and Thus (1993, GLP). The test was in duplicate with activated sludge obtained from a sludge plant predominantly treating domestic waste water. The test substance was dissolved in acetone. The bottles were filled with mineral salt medium, inoculum at a concentration of 30 mg dw/L, and test substance at a concentration of 11.25 μg/l. The bottles were incubated on a shaker at 20°C in the dark for 28 days. CO_2 was trapped in KOH solution and determined by LSC at zero time and after 7, 14, 21, 28 and 29 days. At each sampling 50 ml was extracted with ACN/water and analysed by HPLC with UV and LSC detection to determine possible metabolites.

The recovery by the HPLC analytical method, after spiking the medium with 17 μg/l in triplicate, was 106%. The recovery of ^{14}C from the medium + the CO_2 trap ranged from 82% to 100%. Diflubenzuron was degraded very quickly, the amount in the medium decreasing from 80% at zero time to 11% after 7 days, and at the end of the study to ≤1.5%. The released CO_2 increased to 25% after 28 days. The two major metabolites were CPU and DFBA. CPU was found at a maximum of 51% after 21 days and DFBA at a maximum of 15% after 14 days.

Only bottles with the test substance were incubated, with no negative, positive, or toxicity controls. The results are not considered reliable.

Biodegradation in water/sediment systems. A water/sediment study was conducted with [^{14}C]diflubenzuron (chemical purity 99.1%, radiochemical purity 99.4%, specific activity 721.5 MBq/g, labelled in both phenyl rings) according to Dutch guideline CTB G2.1 (Thus and Van der Laan-Straathof, 1994, GLP). Sediment (from the upper 2-3 cm) and water were collected from two experimental ditches (Heino with sandy loam and Zeewolde with silty loam, both in The Netherlands) and put into aquaria to settle. The ditches were constructed in 1982, filled with soil from elsewhere and covered with water in 1991. The ditches were not treated with pesticides. Sediment was sieved through a 2 mm sieve before putting into the aquarium. Tubes of 100 ml were filled with 7.5 g dw sediment and 67.5 ml pond water. The water was aerated with CO_2-free air without disturbing the sediment. The water/sediment systems were acclimatized at 20°C under a 12:12 hour light-dark regime for 6 weeks. After acclimatization diflubenzuron was added as an aqueous suspension to the water at a rate of 0.94 mg/kg total sediment plus water. The composition and particle size in the suspension was comparable to a WP 250 formulation. Diflubenzuron was mixed homogeneously with the water, taking care not to disturb the sediment. Incubation was in an incubation chamber at 20°C with a 12 hours light/dark cycle. CO_2 and organic volatiles were trapped by KOH and a C-18 cartridge. Three tubes were sampled at 0, 1, 3, 7, 21, and 35 days and two tubes at 45 days. Water and sediment were separated by centrifugation and the radioactivity in the water determined by LSC. Samples of the water were frozen and concentrated by freeze-drying, and the residue was taken up in ACN and water and analysed by HPLC-LSC. Sediment was extracted twice with ACN and twice with methanol with ultra-sonification. After centrifugation the radioactivity in the extracts was measured by LSC, and the extracts analysed by HPLC-LSC after solvent exchange. Bound residues were determined by combustion and LSC. KOH solutions were analysed by LSC, after elution from C-18 columns with ACN. Metabolites were identified by TLC by comparison with reference compounds. For validation water samples of 20 ml were spiked with 10, 50, 100, and 200 μl diflubenzuron suspension and sediment samples with 5, 10, 20, 40, and 100 μl and analysed in the same way.

The mean recovery from the spiked water samples was 92%. Recoveries from spiked sediment samples were 104% for sandy loam and 100% for silty loam. Total experimental recoveries were 95%-104% of the applied radioactivity in the sandy loam system and 92%-100% in the silty loam system.

In sandy loam no volatiles except CO_2 were found. CO_2 reached 4.5% on day 35 and amounted to 3.5% on day 45 (only one analysis). The bound residues increased to 14% on day 35 and amounted to 9.6% on day 45. Radioactivity in the water decreased to 20% on day 21 and increased to 33% on day 45. Radioactivity in sediment increased to a maximum of 68% on day 7 and decreased thereafter to 49% on day 45. DFBA and CPU were found in the water and sediment at maximum amounts in the water phase of 14.9 and 8.91 μg diflubenzuron equivalents respectively after 45 days. This equals 21.1 and 13% of the applied diflubenzuron (70.7 μg). In the sediment, the amount of DFBA was 1.3 μg diflubenzuron equivalents on day 45 (1.8%), when CPU reached a maximum of 11.98 μg equivalents (17%). No other metabolites were found. The authors estimated graphically a half-life of 1.9 days for the water phase and of 25 days for the system.

In silt loam CO_2 reached 5.1% and bound residues 19% on day 45. Radioactivity in the water decreased to 26% on day 7 and increased thereafter to 35% on day 45. Radioactivity in the sediment decreased to 34% on day 35 and was 35% on day 45. DFBA and CPU were found in both water and sediment, with maximum amounts in the water phase of 16 μg diflubenzuron equivalents (23%) after 35 days, and 11.9 μg (17%) on day 21 respectively. In the sediment, the maxima of DFBA and CPU were found on day 35: 6.3 and 47.4 μg diflubenzuron equivalents, equalling 9.0 and 67% of the applied diflubenzuron. Other metabolites were not found. The authors estimated a half-life of 1 day for the water phase and of 10 days for the system.

The authors reported the water solubility of 80 μg/l for diflubenzuron (no temperature or pH mentioned). The amount of diflubenzuron added exceeded this. The results are considered reliable.

The degradation of a mixture of [^{14}C]chlorophenyl-labelled (radiochemical purity >98%, specific activity 5.073 MBq/mg) and [^{14}C]benzoyl-labelled diflubenzuron (radiochemical purity >98%, specific activity 8.217 MBq/mg) in two water-sediment systems was studied according to German BBA, EU and SETAC (Society of Environmental Toxicology and Chemistry) guidelines (Voelkel, 1999, GLP). Water with sediment (loamy sand and loam) were sampled from the river Rhine and a pond respectively, in Switzerland. The sediments were 2 mm-sieved, and a layer of about 2.5 cm (200 g wet weight, corresponding to 117 g dw for the river sediment and 69 g dw for the pond sediment) was added to a 1 l flask (88.2 cm$^{2\ \mathrm{area}}$). A 6 cm layer of the corresponding water was added (550 ml), leading to a dry weight sediment:water ratio of about 21% and 12.5% for river and pond systems respectively. The units were pre-incubated for 52 days under aerobic conditions at 20 ± 2 ˚C in the dark with pH, dissolved oxygen and redox potential monitored, then treated with 55 μg diflubenzuron (equivalent to 100 µg/l based on 0.3 kg ai/ha and 30 cm depth) added as a solution in ACN dropwise on the water surface. The flasks were incubated in the dark at 20 ± 2 °C under aeration with CO_2-free air. Duplicate vessels were sampled after 0 and 4 hours, and 1, 2, 8, 16, 30, 62, and 100/104 days. Additional vessels were prepared in a separate experiment about 3 months later to determine values after 4 days of incubation. Volatiles were trapped in ethylene glycol, and NaOH traps were used to collect CO_2 (a second trap was installed after day 16). The traps were sampled on the sampling days or about every 14 days, whichever was shorter. The redox potential, pH, and dissolved oxygen of the water were monitored with the same frequency.

Water was separated from the sediment with a pipette, 100 ml ACN was added and radioactivity in the surface water was counted by LSC after mixing. A 150 ml sample was concentrated to about 10 ml, 2-4 ml DMSO was added and the samples ultrasonically mixed. 5 ml 2 M HCl was added to samples from days 4, 62 and 104 before the DMSO to release dissolved CO_2. Samples from days 8, 16 and 30 were further concentrated to about half the volume. The recovery after concentration was 90-103%. Residues were analysed by LSC (reported LOQ 0.2 µg/l), HPLC (reported LOQ 0.1 µg/l), and TLC.

Sediment was extracted twice with ACN, once with methanol, water (days 0, 8 and later samples), and by Soxhlet overnight with acetone/water (9:1; samples from day 8 and later). Radioactivity in the individual extracts was counted by LSC (reported LOQ 0.2 µg/kg), and extracts with >1% of the applied radioactivity were pooled and concentrated. Recoveries after extraction were 90% to 109%. Extracts were analysed by HPLC (reported LOQ 0.3 µg/kg). The remaining sediment was counted by LSC after combustion (reported LOQ 0.2 µg/kg). Day 104 samples were additionally extracted under reflux with ACN/2M HCl (80:20), and the extracts analysed by LSC and HPLC. Organic matter in these samples was fractionated by extraction with 0.5 M NaOH and precipitation of humic acid by reducing the pH to 1. Volatiles in ethylene glycol were counted by LSC (reported LOQ 0.02 µg). $^{14}CO_2$ in the NaOH traps was counted by LSC after precipitation with $Ba(OH)_2$ (reported LOQ 0.02 µg). Reference substances were PCAA, CPU, DFBA, DFBAM and PCA. The microbial biomass in the sediment was determined in control vessels at the start and end of the experiment.

In the river system the microbial biomass in the sediment was 0.5 g C/kg dw at the start of the incubation, and 0.9 g C/kg dw at the end. The average pH of the water was between 7.9 and 8.0, the oxygen level was 4.5 to 7.6 mg/l, the redox potential of the water was +147 to +227 mV and of the sediment –6 mV before sampling and –148 mV at the end of the experiment. The mean recovery of radioactivity was 95 ± 3.6% of that applied (range 90-101%). The distribution of radioactivity is shown in Table 43.

Table 43. River system. Distribution of radioactivity.

Days	^{14}C, % of applied[1]					
	Water	Sediment extract	Bound residues	CO_2	Organic volatiles	Total
0	69	24	0.2	-	-	93
0.17	86	12	0.2	<0.1	<0.1	98
1	71	26	1.7	<0.0	<0.1	99
2	72	25	2.1	0.1	<0.1	99
4[2]	69	27	6.0	0.8	0.0	103
8	46	33	9.7	4.6	<0.1	94
16	37	24	16	14	0.1	90
30	27	19	23	24	0.4	93
62	8.6	12	38	32	1.0	92
104	6.4	8.8	44	33	0.6	93

[1] mean of duplicate samples

[2] separate vessels incubated 3 months later

Analysis of the sediment showed that the bound residues were mainly associated with humins. Table 44 shows the compounds identified in the water and sediment as percentages of the applied radioactivity. DFBAM was not found in the sediment. Unknown compounds in the water accounted for a maximum of 2.9% of the applied radioactivity.

Table 44. River system. Compounds identified in water and sediment.

day	^{14}C, % of applied										
	Water				Sediment			System (rounded values)			
	parent	CPU	DFBA	DFBAM	parent	CPU	DFBA	parent	CPU	DFBA	DFBAM
0	69				24			93			
0.17	86	0.3	0.3		11			97	0.3	0.3	
1	68	2.1	1.7		24	1.2	0.2	92	3.3	1.9	
2	63	5.3	4.4		22	2.1	0.2	85	7.4	4.6	
4[1]	41	16	7.3	0.9	17	6.7	3.7	58	23	11	0.9
8	12	29	1.2	2.6	21	10	0.7	33	39	1.9	2.6
16	0.3	31		1.7	8.4	16		8.7	47		1.7
30		24		0.5	3.7	15		3.7	39		0.5
62		6.2		0.6	2.6	8.6		2.6	15		0.6
104		2.8			2.6	5.2		2.6	8.1		

[1] separate vessels incubated 3 months later

In the pond system the microbial biomass in the sediment was 2.7 g C/kg dw at the start of the incubation, and 2.1 g C/kg dw at the end. The average pH of the water was 8.0, the oxygen level was 4.2 to 6.7 mg/l, the redox potential of the water was +136 to +228 mV, and the sediment 0 mV before sampling and –186 mV at the end of the experiment. The mean recovery of radioactivity was 94 ± 3.8% of that applied (range 90%-103%). The distribution of radioactivity is shown in Table 45.

Table 45. Pond system. Distribution of radioactivity.

Day	^{14}C, % of applied					
	Water	Sediment extract	Bound residues	CO_2	Organic volatiles	Total
0	72	21	0.3		-	94
0.17	82	16	0.4	<0.1	<0.1	98
1	81	15	1.7	<0.1	<0.1	98
2	76	20	3.2	0.1	<0.1	98
4[1]	62	32	6.5	0.5	<0.1	100
8	52	24	13	1.8	<0.1	91
16	32	24	20	14	0.2	90
30	20	21	22	30	0.3	32
62	10	18	29	35	0.3	92
104	5.5	11	36	38	0.3	91

Again the bound residues were mainly associated with humins. Table 46 shows the distribution of the identified compounds in the water and sediment. Unknown compounds in the water accounted for a maximum of 0.8% of the applied radioactivity.

Table 46. Pond system. Compounds identified in water and sediment.

day	^{14}C, % of applied											
	water				sediment				system (rounded values)			
	parent	CPU	DFBA	DFBAM	parent	CPU	DFBA	DFBAM	parent	CPU	DFBA	DFBAM
0	72				21				93			
0.17	82				15				97			
1	77	2.4	1.7		9.2	3.6	1.3		86	6.0	3.1	
2	64	6.4	5.5		12	5.5	1.2		76	12	6.7	
4[1]	29	18	13	1.2	18	9.5	2.6	0.2	47	27.5	17	1.4
8	11	31	8.6	0.5	5.0	16	0.5		16	47	11	0.5
16	0.3	28		2.4	2.0	20		0.1	2.3	48	0.5	2.4
30		20				21				41		

day	^{14}C, % of applied											
	water				sediment				system (rounded values)			
	parent	CPU	DFBA	DFBAM	parent	CPU	DFBA	DFBAM	parent	CPU	DFBA	DFBAM
62		10				18		0.3		28		0.3
104		4.9		0.2		10		1.1		15		1.3

[1] separate vessels incubated 3 months later

The authors calculated the degradation rates shown in Table 47.

Table 47. Degradation rates of diflubenzuron, CPU and DFBA in river and pond water/sediment systems.

	River, days	Pond, days
diflubenzuron		
DT-50, water	3.2	2.8
DT-90, water	11	9.4
r^2	0.997	0.994
kinetics	first order	first order
DT-50, system	5.4	3.7
DT-90, system	18	12
r^2	0.995	0.995
kinetics	first order	first order
CPU		
DT-50, water	18	32
DT-90, water	60	106
r^2	0.985	0.975
kinetics	consecutive reaction	consecutive reaction
DT-50, system	27	52
DT-90, system	89	174
r^2	0.998	0.991
kinetics	consecutive reaction	consecutive reaction
DFBA		
DT-50, water	1.5	4.2
DT-90, water	5.0	14
r^2	0.999	0.981
kinetics	first order	first order
DT-50, system	1.6	4.4
DT-90, system	5.2	15
r^2	0.999	0.984
kinetics	first order	first order

Half-lives of DFBA are not considered reliable. The graphs show that data from 0 to 2 days are ignored and that fits are based on samples from days 4 and 8, all other values being taken as zero. Half-lives of diflubenzuron, and information on bound residues, CO_2 production and metabolite formation are considered reliable. Half-lives of CPU should be regarded as indicative.

An anaerobic water/sediment study was conducted with [^{14}C]diflubenzuron (radiochemical purity 99.0%, chemical purity 100.2%, specific activity 9.91 mCi/g), labelled in both phenyl rings, according to EPA guideline 162-3 (Thus *et al.*, 1991, Thus and Van Dijk, 1991, GLP). Sediment (from the upper 5 cm) and water were collected from the same pond as in the previous study. The sediment was sieved through a 2 mm sieve. Tubes containing 3.5 g dw sediment and 36.5 g pond water were acclimatized in the dark in a climate room at 24°C for four weeks. After the acclimatization period, the total weight of the sediment and water was reduced to 35 g by removing water. Diflubenzuron was added to the water as an aqueous suspension at a dosage of 1.3 mg/kg based on the weight of the sediment and water. The composition and particle size in the suspension were comparable to a WP 250 formulation. The tubes were incubated in a nitrogen stream without disturbing the sediment for 42 days. Volatiles were trapped by KOH and ethylene glycol. Three tubes were sampled at 0 hours, and 4, 7, 11, 14, 18, 21, 28, 35, and 42 days. The experiment was subsequently prolonged to 90 days in total. Water and sediment were separated by centrifugation and the radioactivity in the water layer was determined by LSC. The sediment was extracted three times with methanol and the radioactivity of the extracts measured by LSC. Water and sediment extracts were analysed by HPLC-LSC and TLC. Bound residues were counted by combustion and LSC.

The HPLC-LSC analysis was validated in three ways. 1) Blank sediment and water were spiked separately with diflubenzuron at 0-1 mg/l. 2) A sediment/water system was spiked with levels of diflubenzuron ranging from 0 to 1 mg/l in the total system. 3) At the beginning of the study, nine tubes with the sediment/water system were treated with three different amounts of diflubenzuron and processed in the same way as the other tubes.

The mean recovery of diflubenzuron from spiked sediment was 96% (n=10), and from spiked water 98% (n=9). The response of the whole procedure was linear over the concentration range of 0-1 mg/l (r=0.997 for water samples and r=0.998 for sediment samples). The mean recovery for the whole procedure was 93% (n=8). The recovery of diflubenzuron during concentration and analysis in the method validation experiment was 98% (n=9) for the sediment and 97% (n=8) for the water. During the whole study, the recovery for concentration and analysis was 98% (n=35) for the sediment and 97% (n=35) for the water.

The oxygen content of the water ranged from 0.5 mg/l at zero time to 0.2 mg/l on day 42, and its pH from 7.3 at zero time to 8.3 on day 42. pH and oxygen were measured just above the sediment layer.

During the first phase, days 0-42, the total recovery ranged from 96% to 99% of the applied radioactivity. No volatiles except CO_2 were measured. CO_2 increased to 0.95% on day 42. The radioactivity in the sediment decreased from 54% on day 0 to 36% on day 42, and in the water increased from 42% on day 0 to 56% on day 42. The bound residues increased from 1% on day 0 to 4.3% on day 35 and amounted to 3.7% on day 42. In the water phase diflubenzuron decreased from 40% on day 0 to 10% on day 42, and in the sediment from 52% on day 0 to 26% on day 42. Both DFBA and CPU were found in the water and sediment. On day 42 DFBA reached a maximum of 26% in the water and was about 2% in the sediment, while CPU also reached its maximum in the water layer (21%) on day 42, when it amounted to 8.4% in the sediment. The authors calculated a half-life of diflubenzuron of 18 days for the water and 34 days for the whole system with a first order kinetics model.

After 90 days $^{14}CO_2$ accounted for 2.8% of the applied ^{14}C, and diflubenzuron for 1.8% in the system, with <1% in the water and 1.6% in the sediment. The radioactivity was 66% of that applied in the water and 20% in the sediment. DFBA and CPU were the only products found, at levels of 39% and 26% in the water and 5.5 and 13% in the sediment. The degradation followed first order kinetics during the first 56 days but was faster thereafter, probably by adaptation of the micro-organisms. The author estimated half-lives of 34 days in the water and the whole system.

The amount of diflubenzuron added to the water layer exceeds the water solubility. The half-lives of diflubenzuron recalculated by linear regression of log concentrations versus time, during the first 56 days ($r^2 \geq 0.9$), were 28 days for the water phase and 48 days for the system (20°C). The results are considered reliable.

Biodegradation in water. A mixture of [^{14}C]benzoyl-labelled diflubenzuron (specific activity 8.68 mCi/mmole; radiochemical purity ≥99.3%) and [^{14}C]chlorophenyl-labelled diflubenzuron (specific activity 8.3 mCi/mmole, radiochemical purity ≥99.1%) was added to freshly sampled ditch water from an unpolluted ditch at Nederhorst den Berg in The Netherlands (Nimmo and De Wilde, 1975, non-GLP). The diflubenzuron was dissolved in acetone and 1 ml added to 1.8 l water in 2 l Erlenmeyer flasks, to give a rate of 0.1 mg/l. The solutions were incubated under gentle aeration for 0, 14, 28, 56 or 84 days, and the flasks connected to wash bottles containing ethanolamine to trap volatiles; further details not provided. Water samples (300 ml) were taken for analysis and total radioactivity was determined by LSC. The water was extracted with ether and n-butanol and analysed for diflubenzuron, DFBA and CPU by TLC, MS and reversed isotope dilution analysis.

The total recovery of radioactivity as determined by LSC was 100-110%. Degradation of diflubenzuron followed first order kinetics, with 9% remaining after 84 days. Significant amounts of DFBA (33 and 34%) and CPU (38% and 42%) were recovered by days 56 and 84 respectively. Unidentified radioactivity amounted to 0, 20 and 18% on days 0, 56 and 84. After 56 days, 1% of the applied activity was recovered from the volatile traps.

The half-life recalculated by applying linear regression to log concentrations versus time is 22 days (r^2=0.98).

The microbial and chemical degradation of diflubenzuron (purity ≥99.5%) was determined in freshly sampled water from a canal in 's Graveland to generate input for the EXAMS program of the EPA (Van Dijk, 1994, GLP). The water was filtered through glass wool and transferred to a 2 l Erlenmeyer flask, which was sealed and pre-incubated for 3 days at 25 ± 1°C in the dark. After incubation, 100 ml water was put into eight 250 ml Erlenmeyer flasks, two of which were sterilized by autoclaving for 15 min at 121°C. Diflubenzuron was dissolved in acetone and 4 to 12 µl was added to the flasks to reach final fortification rates of 19.2 to 57.6 µg/l. The concentration in the sterilized water was 57.6 µg/l; all flasks were prepared in duplicate. The solutions were incubated at 25°C. Water samples for chemical analysis were taken at 0, 1, 6, 14 and 17 days, those from the sterilized flasks under aseptic conditions. Water was analysed by HPLC after ultrasonification for 10 min followed by centrifugation at 16000 g. At 0 and 17 days, duplicate 0.1 ml samples from sterilized and unsterilized flasks containing 57.6 mg/l were taken for microbial analysis: samples from the sterilized flasks were applied directly to agar plates, and the unsterilized samples were diluted first in 20 ml sterilized water. The plates were incubated for 5 days at 25°C and counted.

The initial recovery was 99 to 125% of the nominally added diflubenzuron from the non-sterile samples, and 76 and 102% from the sterile samples. At the end of the experiment after 17 days, 85 and 109% of the initially determined amount was recovered from the sterile samples. The half-life of diflubenzuron in all the non-sterile samples was about 10 days according to the authors.

Microbial analysis of non-sterile samples showed 54 and 42 CFUs initially from one flask and >100 and 55 from the other. The sterile samples yielded 0 and 1 CFU. After 17 days, there were 26 to 51 CFUs in the non-sterile samples, 0 in one of the sterile replicates and >100 in the other. The author stated that the samples with >100 CFUs were infected because the plates were placed too close to a ventilator.

The microbial assay was not reliable because of non-sterile conditions. Half-lives recalculated assuming first order kinetics were 7.8 and 12.5 days at 19.2 µg/l, 6.1 and 12.4 days at 38.4 µg/l, and 7.6 and 4.9 days at 57.6 µg/l. The overall average was 8.6 ± 3.2 days (equivalent to about 13 days at 20°C). This result is considered reliable.

Effect of spray drift. The EPA computer modelling programs PRZM-2 and EXAMS II were used to model the fate of diflubenzuron when applied to a citrus grove located 33.5 m from a 1 hectare, two metre deep pond (Harned and Relyea, 1997). From the results it is clear that spray drift is the main route of introduction of diflubenzuron to the adjacent water and that without drift mitigation measures there will be an acute risk to aquatic organisms.

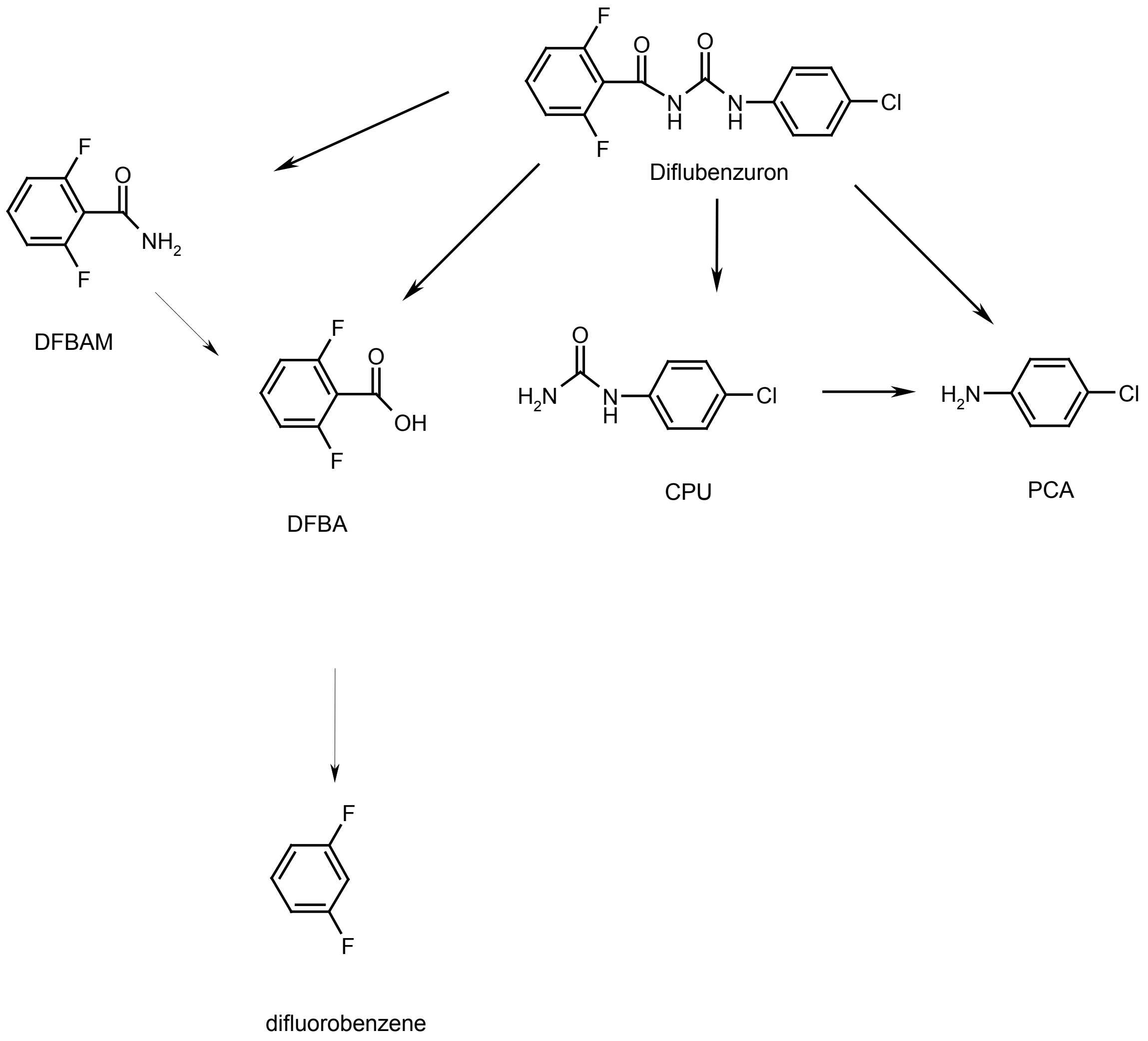

Figure 3. Breakdown of diflubenzuron in soil and water.

RESIDUE ANALYSIS

Analytical methods

Many analytical methods used in supervised residue trials or in studies on storage stability, processing, animal feeding or direct animal treatment were reported. Most are single-residue methods for either diflubenzuron, DFBA, CPU, PCA or PCAA in only a few substrates. HPLC methods for diflubenzuron, CPU or PCAA consist of extraction, clean-up and determination with UV, MS or MS-MS detection. GC methods for diflubenzuron, CPU or PCA consist in hydrolysis of PCA conjugates, extraction, clean-up, and hydrolysis of diflubenzuron, followed by derivatization with HFBA and determination with EC or MS detection. In GC methods for DFBA extraction is followed by clean-up and derivatization with pentafluorobenzyl bromide (PFBBr) or diazomethane and determination by GC-ECD or GC-MS.

LOQs quoted in the Tables are those stated by the authors of the reports, whether supported by recoveries from spiked samples at that level or not.

Methods proposed for enforcement

Enforcement methods for the separate determination of diflubenzuron, CPU or PCA in rice were reported (method LAI-3-86-6 for diflubenzuron, LAI-3-86-9 for CPU and PTRL 625W for PCA).

The Meeting was also informed of the existence of three enforcement methods used in the USA and published in the Pesticide Analytical Method Volume II (PAM II): GC-ECD methods for the separate determination of diflubenzuron, CPU or PCA in eggs, milk and livestock tissues and a method for the determination of diflubenzuron in pears. Details and validation results were however not submitted.

HPLC methods for diflubenzuron, CPU or PCAA

HPLC methods for the determination of diflubenzuron, CPU or PCAA in plant products, animal products or soil are based on extraction followed by a suitable clean-up procedure and direct determination by HPLC-UV, LC-MS or LC-MS-MS. Methods for the determination of diflubenzuron in water involve clean-up followed by direct determination by HPLC-UV.

With HPLC-UV methods suppressive matrix effects are extremely unlikely but interference is very likely. Significant under-estimates of true recovery are very unlikely and in the absence of interferences over-estimates are also unlikely. Except at the extremes of the response range, the linearity of UV absorption is inherently good so in principle the reported recoveries are likely to be realistic, but there is evidence of frequent interference with low-concentration measurements.

The UV spectrum of diflubenzuron is very similar to that of many other aromatic compounds (including its metabolites) and the resolution of most HPLC columns is not particularly good. Apparent outliers among data for control samples should not be discarded unless the samples are proved to have been contaminated with diflubenzuron (e.g. by identification with MS).

Interference is also observed in HPLC-MS methods. A single MS-MS transition does not provide good specificity in all cases. If the two isotopic forms (^{35}Cl and ^{37}Cl) of the diflubenzuron or CPU molecule are monitored and the transitions provide responses in the correct ratios, then specificity is considered adequate.

HPLC-UV methods for diflubenzuron

Method LAI 3-86-2, version of 27 May 1975 (Philips-Duphar, 1975, non-GLP) described the determination of diflubenzuron in apples. Samples (100 g) were extracted by macerating with dichloromethane, anhydrous sodium sulfate and filter pulp (to break the emulsion). The filtered extract was evaporated to dryness, redissolved in dichloromethane/petroleum ether (3+25), purified on a Florisil column, evaporated to dryness and redissolved in DMSO/water (1+1). Diflubenzuron was determined by HPLC-UV (Corasil C-18, mobile phase methanol/water (6+4), 254 nm) with external standards. The method was used for apples, pears, head cabbage, tomatoes and mushrooms.

De Wilde (1978d, non-GLP) modified the method for Brussels sprouts by adding an extra clean-up procedure as described in method LAI-3-86-4 which itself was not available, and for compost mushroom casing (1978a,b, non-GLP) by omitting the filter pulp and changing the amount of sodium sulfate and sample (50 g).

In the method for blackcurrants, gooseberries and tomatoes (De Wilde, 1980, non-GLP) the amount of sample was changed and samples were extracted with ethyl acetate instead of dichloromethane.

The method was modified in 1982 for apples and pears (LAI 3-86-2; 2 August 1982). The mobile phase was changed to dioxane/water/ACN (10 + 45 + 45) and the residue was dissolved in the mobile phase. The full description was not submitted.

Buisman and Snijders (1987b) modified this version of the method for chicken manure by directly adding petroleum ether to 3 ml of the dichloromethane extract

The method was again modified as LAI 3-86-2 of 24 February 1988 (Solvay Duphar, 1991, non-GLP). Differences from the 1982 method were not indicated. Pouwelse and Van Zijtveld (1991, GLP) used this method for tomatoes. In control samples a small interference peak with a retention time close to diflubenzuron was found (max 0.018 mg/kg diflubenzuron). The calibration curve in the range 0.2-1.0 mg/l was linear (6 single points, including blank) with a regression coefficient r>0.99999.

Validation reports were not available for the original method or the modifications. The methods were validated at the time of supervised trials with a limited number of recoveries. The results are shown in Table 48. Selectivity in the presence of DFBA, CPU and PCA was not verified for mushrooms, compost or chicken manure.

Table 48. Recoveries of diflubenzuron from spiked samples by method LAI 3-86-2 and its modifications.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean	% Rec range	% RSD$_r$	Controls, mg/kg (no. of samples)	Reference (type of trial)
LAI 3-86-2, May 1975								Philips-Duphar, 1975
apples	0.03	0.03 0.05 0.06 0.1 0.3 0.5 0.6 1 2	3 2 1 3 3 3 1 2 3	62 116 73 94 94 101 100 102 103	52-77 71-160 - 84-112 87-98 94-108 - 102-103 99-107	21 - - 17 6.5 9.8 - - 3.9	<0.009-0.094 (55)	Buisman *et al.*, 1975, non-GLP (residue trial, DI-2984)
apples	0.03	0.03 0.05 0.1 0.3 0.5 1 2	1 2 1 1 2 2 1	107 101 88 97 104 116 100	- 82-120 - - 102-106 105-127 -	- - - - - - -	<0.009-0.34 (10)	Buisman *et al.*, 1976e, non-GLP (residue trial, DI-2723)
apples	0.03	0.03 0.05 0.06 0.1 0.3 0.5 1 2	6 2 3 2 2 3 2 2	69 86 56 77 94 93 100 104	36-97 77-94 29-87 77-77 85-102 86-104 96-103 101-106	31 - 53 - - 10 - -	<0.009-0.12 (15)	Buisman *et al.*, 1976c, non-GLP (residue trial, DI-1253)
apples	0.03	0.03 0.05 0.06 0.1 0.3 0.5 0.6 1 2	2 1 3 5 1 1 2 2 1	106 92 81 81 76 90 102 108 113	69-106 - 57-100 75-88 - - 99-105 100-115 -	- - 27 9.2 - - - - -	<0.009-0.096 (32)	Buisman *et al.*, 1976b non-GLP (residue trial, DI-1249)
apples	0.05	0.05 0.1 0.3	1 1 1	100 120 80	- - -	- - -	<0.015 (9)	Buisman *et al.*, 1976f, non-GLP (residue trial, DI-2725)

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean	% Rec range	% RSD$_r$	Controls, mg/kg (no. of samples)	Reference (type of trial)
		0.5	1	103	-	-		
		1	1	105	-	-		
		2	1	112	-	-		
apples	0.03	0.03	2	72	48-97	-	<0.009-0.072 (17)	Buisman *et al.*, 1976d, non-GLP (residue trial, DI-2403)
		0.06	2	102	73-131	-		
		0.1	2	79	70-88	-		
		0.3	2	99	89-109	-		
		0.5	2	94	94-95	-		
		1	2	100	96-104	-		
		2	2	100	95-106	-		
apples	0.01	0.01	2	90	60-120	-	<0.003-0.89 (4)	De Wilde, 1977e, non-GLP (residue trial, DI-2259)
		0.03	2	106	93-120	-		
		0.05	2	93	82-104	-		
		0.1	2	87	74-100	-		
		0.3	2	179	77-102	-		
		0.5	2	98	95-100	-		
		1	2	78	48-108	-		
		2	2	100	99-101	-		
		5	1	108	-	-		
apples	0.01	0.01	1	120	-	-	0.008-0.080 (6)	De Wilde, 1977f, non-GLP (residue trial, DI-2260)
		0.03	1	64	-	-		
		0.05	1	92	-	-		
		0.1	2	75	58-92	-		
		0.5	1	99	-	-		
		1	1	101	-	-		
		2	1	98	-	-		
apples	0.01	0.01	1	80	-	-	<0.003-0.024 (23)	De Wilde, 1977g, non-GLP (residue trial, DI-2272)
		0.03	1	67	-	-		
		0.05	1	80	-	-		
		0.08	1	98	-	-		
		0.1	1	96	-	-		
		0.3	1	100	-	-		
		0.5	1	99	-	-		
		0.8	1	102	-	-		
		1	1	103	-	-		
		2	1	99	-	-		
		3	1	98	-	-		
		5	1	95	-	-		
apples	0.03	0.03	1	87	-	-	<0.009-1.0 (11)	De Wilde, 1977h, non-GLP (residue trial, DI-2273)
		0.05	1	82	-	-		
		0.1	1	100	-	-		
		0.3	1	96	-	-		
		0.5	1	97	-	-		
		1	1	96	-	-		
		2	2	94	-	-		
apples	0.03	0.03	1	87	-	-	<0.009-0.027 (18)	De Wilde, 1977c, non-GLP (residue trial, DI-901)
		0.04	1	73	-	-		
		0.08	1	74	-	-		
		0.3	1	92	-	-		
		0.5	1	90	-	-		
		1	1	100	-	-		
		2	1	104	-	-		
apples	0.01	0.01	1	80	-	-	<0.003-0.067 (11)	De Wilde, 1978c, non-GLP (residue trial, DI-1304)
		0.03	1	106	-	-		
		0.05	1	104	-	-		
		0.1	1	93	-	-		
		0.3	1	91	-	-		
		0.5	1	96	-	-		
		1	1	102	-	-		
		2	1	102	-	-		
apples	0.03	0.03	1	72	-	-	0.004-0.10 (6)	De Wilde, 1979b, non-GLP (residue trial, DI-634)
		0.05	1	89	-	-		
		0.1	1	88	-	-		
		0.3	1	109	-	-		

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean	% Rec range	% RSD$_r$	Controls, mg/kg (no. of samples)	Reference (type of trial)
		0.5	1	100	-	-		
		1.0	1	104	-	-		
		3.0	1	104	-	-		
		5.0	1	99	-	-		
apples	0.01	0.01	1	117	-	-	<0.003-0.017 (6)	De Wilde, 1980, non-GLP (residue trial, DI-201)
		0.04	1	69	-	-		
		0.06	1	93	-	-		
		0.1	1	87	-	-		
		0.4	1	91	-	-		
		0.6	1	98	-	-		
		1.2	2	102	101-103	-		
		2.4	1	98	-	-		
apples	0.05	0.05	1	98	-	-	<0.015-0.034 (3)	Buisman, 1980, non-GLP (residue trial, DI-129)
		0.1	1	116	-	-		
		0.3	1	110	-	-		
		0.5	1	93	-	-		
		1.0	1	109	-	-		
		2.0	1	104	-	-		
apples	0.1	0.1	2	98	92-104	-	<0.03-0.048 (15)	De Wilde, 1984, non-GLP (residue trial, DI-4292)
		0.6	5	101	93-108	5.3		
		1	5	93	90-97	3.0		
apples	0.03	0.03	1	107	-	-	<0.009 (8)	De Wilde, 1982, non-GLP (residue trial, DI-779)
		0.05	1	84	-	-		
		0.1	1	88	-	-		
		0.3	1	98	-	-		
		0.5	1	100	-	-		
		1.0	1	110	-	-		
		2.0	1	103	-	-		
pears	0.03	0.03	1	90	-	-	<0.009-0.012 (5)	De Wilde, 1977b, non-GLP (residue trial, DI-2286)
		0.05	1	100	-	-		
		0.1	1	85	-	-		
		0.3	1	109	-	-		
		0.5	1	122	-	-		
		1.0	1	121	-	-		
		2.0	1	129	-	-		
pears	0.01	0.01	1	90	-	-	0.004-0.020 (8)	De Wilde, 1977a, non-GLP (residue trial, DI-1314)
		0.03	1	87	-	-		
		0.05	1	104	-	-		
		0.1	1	101	-	-		
		0.3	1	98	-	-		
		0.5	1	98	-	-		
		1.0	1	106	-	-		
		2.0	1	92	-	-		
pears	0.01	0.01	1	100	-	-	0.010 (2)	De Wilde, 1977d, non-GLP (residue trial, DI-2022)
		0.03	1	100	-	-		
		0.1	1	100	-	-		
		0.3	1	95	-	-		
		1.0	1	100	-	-		
pears	0.01	0.01	2	64	55-73	-	<0.003-0.054 (21)	De Wilde, 1977c, non-GLP (residue trial, DI-901)
		0.03	2	62	35-88	-		
		0.05	2	102	100-104	-		
		0.1	2	71	47-95	-		
		0.3	2	82	76-89	-		
		0.5	1	90	-	-		
		1.0	2	98	94-103	-		
		2.0	1	93	-	-		
pears	0.04	0.04	2	77	70-84	-	<0.012-0.049 (9)	Buisman, 1981, non-GLP (residue trial, DI-875)
		0.06	1	89	-	-		
		0.1	1	76	-	-		
		0.4	2	84	80-88	-		
		0.6	1	98	-	-		
		1	1	99	-	-		
head cabbage	0.03	0.03	2	94	80-107	-	<0.009-0.47 (49)	Buisman *et al.*, 1976g non-GLP
		0.04	1	85	-	-		

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean	% Rec range	% RSD$_r$	Controls, mg/kg (no. of samples)	Reference (type of trial)
		0.05	2	76	64-88	-		(residue trial, DI-4334)
		0.07	1	70	-	-		
		0.1	1	80	-	-		
		0.3	2	98	91-105	-		
		0.5	4	92	72-105	16		
		1	3	96	81-110	15		
		2	3	92	90-95	2.7		
tomatoes	0.03	0.03	1	103	-	-	<0.009 (4)	De Wilde, 1978f, non-GLP (residue trial, DI-4345)
		0.05	1	110	-	-		
		0.1	2	102	96-107	-		
		0.3	1	97	-	-		
		0.5	2	94	92-96	-		
		1	2	98	92-104	-		
		2	2	101	98-104	-		
mushrooms	0.03	0.03	1	59	-	-	<0.009-0.020 (7)	De Wilde, 1978b, non-GLP (residue trial, DI-3410)
		0.06	1	75	-	-		
		0.1	1	85	-	-		
		0.3	1	98	-	-		
		0.6	1	103	-	-		
		1	1	103	-	-		
mushrooms	0.05	0.05	3	59	46-77	27	0.024-0.040 (3)	Buisman *et al.*, 1976a non-GLP (residue trial, DI-2726)
		0.1	2	98	75-120	-		
		0.3	2	126	124-128	-		
		0.5	1	87	-	-		
		2	1	89	-	-		
mushrooms	0.02	0.02	3	53	43-65	21	<0.006-0.009 (11)	De Wilde, 1978a, non-GLP (residue trial, DI-1309)
		0.04	2	76	55-98	-		
		0.06	3	88	87-89	1.1		
		1	1	87	-	-		
		2	1	85	-	-		
mushrooms	0.01	0.01	3	97	80-120	21	<0.003-0.012 (15)	De Wilde, 1979a, non-GLP (residue trial, DI-3409)
		0.04	1	100	-	-		
		0.06	2	102	97-108	-		
		0.1	2	100	95-105	-		
		0.3	1	88	-	-		
		0.5	1	90	-	-		
		0.6	2	86	81-92	-		
		1	2	94	88-100	-		
		2	2	94	92-96	-		
modification A								De Wilde, 1978d
Brussels sprouts	0.02	0.02	1	114	-	-	<0.006-0.058 (5)	De Wilde, 1978d, non-GLP (residue trial, DI-1315)
		0.04	1	76	-	-		
		0.1	2	84	74-94	-		
		0.4	2	80	66-95	-		
		1	1	90		-		
		2	1	98		-		
modification B								De Wilde, 1978a,b
compost	0.1	0.1	1	65	-	-	0.075-0.47 (8)	De Wilde, 1978a, non-GLP (residue trial, DI-1309)
		0.3	1	24	-	-		
		0.5	1	79	-	-		
		1	2	72	64-80	-		
		2	2	94	93-94	-		
		3	1	98	-	-		
		10	1	101	-	-		
		46	1	101	-	-		
		73	1	100	-	-		
		151	1	100	-	-		
		229	1	101	-	-		
compost	0.1	0.1	1	78	-	-	0.080-0.36 (10)	De Wilde, 1978b, non-GLP (residue trial, DI-3410)
		0.2	1	85	-	-		
		0.7	1	96	-	-		
		1	1	88	-	-		
		2	1	95	-	-		
		10	1	92	-	-		

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean	% Rec range	% RSD$_r$	Controls, mg/kg (no. of samples)	Reference (type of trial)
		30	1	83	-	-		
		50	1	96	-	-		
		101	1	89	-	-		
modification C								De Wilde, 1980
gooseberries	0.01	0.01	1	90	-	-	<0.003 (3)	De Wilde, 1980, non-GLP (residue trial, DI-201) *ad hoc* modification: Mar 1980
		0.04	1	88	-	-		
		0.1	1	97	-	-		
		0.4	1	97	-	-		
blackcurrants	0.01	0.01	1	70	-	-	<0.003 (1)	
		0.06	1	80	-	-		
		0.1	1	80	-	-		
		0.4	1	99	-	-		
		1.0	1	98	-	-		
		2.0	1	102	-	-		
tomatoes	0.01	0.01	1	100	-	-	0.005-0.039 (2)	
		0.03	1	107	-	-		
		0.05	1	112	-	-		
		0.3	1	106	-	-		
		0.5	1	109	-	-		
		1.0	1	110	-	-		
		2.0	1	107	-	-		
modification D								LAI 3-86-2: 2 Aug 1982
apples	0.01	0.01	1	91	-	-	<0.003-0.004 (4)	Buisman, 1983, non-GLP (residue trial, DI-4309)
		0.03	1	91	-	-		
		0.1	1	99	-	-		
		0.3	1	94	-	-		
apples	0.03	0.03	2	96	88-103	-	<0.009-0.034 (10)	De Wilde and Buisman, 1985a,b, non-GLP (residue trials DI-4845, DI-4842)
		0.05	1	106	-	-		
		0.1	1	91	-	-		
		0.3	1	101	-	-		
		0.5	1	101	-	-		
		1	1	101	-	-		
		2	1	104	-	-		
apples	0.01	0.01	1	90	-	-	<0.003 (4)	Buisman and Verhaar, 1985 (residue trial, DI-4846) non-GLP
		0.03	1	90	-	-		
		0.05	1	88	-	-		
		0.1	1	97	-	-		
		0.3	1	88	-	-		
		0.5	1	100	-	-		
		1	1	106	-	-		
		2	1	108	-	-		
apples	0.01	0.01	1	110	-	-	<0.003-0.006 (6)	Buisman and Snijders, 1987c (residue trial, DI-6155) non-GLP
		0.05	1	116	-	-		
		0.1	1	99	-	-		
		0.3	1	96	-	-		
		0.5	1	97	-	-		
		1	1	107	-	-		
pears	0.01	0.01	1	80	-	-	<0.003 (4)	Buisman, 1984 (residue trial, DI-4381) non-GLP
		0.03	1	100	-	-		
		0.05	1	86	-	-		
		0.3	1	94	-	-		
		0.5	1	117	-	-		
		1.0	1	103	-	-		
		2.0	1	108	-			
pears	0.01	0.01	1	100	-	-	<0.003 (3)	Buisman, 1983, non-GLP (residue trial, DI-4309)
		0.03	1	103	-			
		0.1	1	102	-			
		0.32	1	101	-			
pears	0.1	0.1	1	102	-	-	0.081-0.16 (6)	Buisman and Snijders, 1987a non-GLP (residue trial, DI-6142)
		0.3	1	101	-	-		
		0.5	1	102	-	-		
		1	1	100	-	-		

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean	% Rec range	% RSD$_r$	Controls, mg/kg (no. of samples)	Reference (type of trial)
modification E								Buisman and Snijders, 1987b
chicken manure	ns	2, 3, 4, 5, 6, 7	1	74-115	-	-	0.000-0.043 (5)	Buisman and Snijders, 1987b non-GLP (storage stability, DI-6200)
modification F								Solvay Duphar, 1991 LAI 3-86-2: 24 Feb 1988
tomatoes	0.03	0.1 0.5 1	1 1 1	96 96 98	- - -	- - -	<0.009-0.018 (5)	Pouwelse and Van Zijtveld, 1991, GLP (residue trial, storage stab., DI-8333)

ns: not stated

Method LAI-3-86-3 (13 January 1976) was used for head cabbage in the residue trials, but a description of this version was not available. A later version (24 February 1988) for the determination of diflubenzuron in cabbage was described by Duphar (1988a, non-GLP). It differed from 3-86-2 in that the evaporated Florisil column eluate was redissolved in dioxane/water (6+4) for HPLC on a Zorbax C-8 column.

A validation report was not available. The method was validated during trials with cabbages with the results shown in Table 49.

Table 49. Recoveries of diflubenzuron from head cabbage by method LAI 3-86-3.

LOQ, mg/kg	Spike, mg/kg	No.	% Rec	Controls, mg/kg	Reference
0.01	0.01 0.03 0.06 0.1 0.3 0.4	1 1 1 1 1 1	82 82 80 63 74 72	<0.003-0.012 (5 samples)	De Wilde, 1980, non-GLP (residue trial, DI-201)

The Japanese HPLC-UV method (Goto, 1977, non-GLP) described the determination of diflubenzuron in apples. The homogenized sample (50 g) was shaken for 30 min with acetone, the extract was filtered, evaporated to dryness and redissolved in n-hexane, partitioned into ACN and evaporated to dryness. The residue was redissolved in n-hexane, purified on a silica gel column, and the eluate evaporated to dryness and redissolved in methanol for analysis by HPLC (Hitachi gel 3050; mobile phase methanol/water (3+2); 258 nm) with external standards in methanol. No validation report was available. Two spiked control samples were analysed during a trial on apples. The results are shown in Table 50.

Table 50. Recoveries of diflubenzuron from apples by Japanese HPLC-UV method.

LOQ, mg/kg	Spike mg/kg	No	% Rec mean.	Controls mg/kg	Reference
0.008	0.8	2	81 (68-93)	0.009-0.014 (4 samples)	Goto, 1977; non-GLP (residue trial, DI-914)

Rabenort *et al.* (1978, non-GLP) described the Brazilian HPLC-UV method for the determination of diflubenzuron in Savoy cabbage, oxheart cabbage, spinach, mushrooms, potatoes, soya beans, ditch mud, ditch water, and milk. Crop samples (100 g) were blended with dichloromethane, anhydrous sodium sulfate and filter pulp. The filtered extract was purified on a Florisil column, evaporated to dryness and redissolved in mobile phase for HPLC (RP-18 column, mobile phase dioxane-water (3+2), UV 254 nm) with external standards.

There was no validation report. Spiked sample of tomatoes were analysed during a trial (Table 51).

Table 51. Recovery of diflubenzuron from tomatoes by the Brazilian HPLC-UV method.

LOQ, mg/kg	Spike, mg/kg	No.	% Rec	Controls mg/kg	Reference
0.02	0.02 0.05 0.1 1	1 1 1 1	98 98 82 96	<0.02	Yokomizo *et al.*, 1992, non-GLP (residue trial, DI-8874)

Method RES010 (6 Oct 1983) was applied to the determination of diflubenzuron in apples and pears and their processed products (ABC, 1984, non-GLP). Homogenized samples (50-100 g) were blended with dichloromethane, anhydrous sodium sulfate and Celite filter aid. The filtered extract was evaporated, redissolved in dichloromethane/petroleum ether (3+25), purified on a Florisil column and evaporated as before, then redissolved in mobile phase and filtered through a 0.22 µm filter. Diflubenzuron was determined on a C-8 column (mobile phase dioxane-water-ACN, 10+45+45; 254 nm) with external standards.

Analyses were validated only during processing trials (Table 52). An interfering peak was found in control samples of apples and apple pomace (wet and dry), which interfered at the 0.05 mg/kg level. The LOQ was not reported.

Table 52. Recoveries of diflubenzuron from apple and pear samples by method RES010. Numbers in parentheses are numbers of control samples.

Sample	Spike, mg/kg	No.	% Rec	Controls, mg/kg	Reference
apples	0.05 0.5 1.5	1 1 1	136 98 100	<0.05 (3)	ABC, 1984, non-GLP (processing trial, DI-4693)
apple juice	0.05 1	1 1	100 93	<0.05 (2)	
wet pomace	0.05 2	1 1	76 80	<0.05 (2)	
dry pomace	0.5 2	1 1	122 95	<0.5 (2)	
apple sauce	0.05 1.5	1 1	80 100	<0.05 (2)	
apple butter	0.5 2	1 1	74 90	<0.5 (2)	
pears	0.05 1.5	1 1	100 100	<0.05 (3)	
canned pears	0.05 1.5	1 1	80 93	<0.05 (2)	

Method LAI 3-86-13, 02 March 1984 (Duphar, 1984a, non-GLP) incorporated a modification for the determination of diflubenzuron in pecan nuts. Homogenized samples (50 g) were blended with ethyl acetate. The filtered extract was evaporated to dryness and the residue dissolved in hexane. After partitioning into ACN and removal of fat and oil by hexane, the ACN extract was evaporated to dryness and the analysis completed by RES010 (no 2 µm filtration). A later description of the method (08 March 1988, Duphar, 1988b) does not differ from the original. The method was used for cotton seed and soya beans.

Thiem (1993, GLP) showed that the calibration curve was linear (r>0.999, 6 single standards including blank in the range 0-80 ng in mobile phase).

Rose (1998a, GLP) modified method LAI 3-86-13 *ad hoc* for mushrooms by omitting the defatting step, as did Kane *et al.* (1995, GLP) who named it CARDC-1248-DFB. Mushrooms were blended with ethyl acetate and anhydrous sodium sulfate. The filtered extract was evaporated to dryness and the residue dissolved in dichloromethane and purified on a Florisil column. The HPLC column was protected by an RP-8 guard column. The method was also used for mushrooms and soya bean forage and hay.

Rose (1999d, GLP) adapted method LAI-3-86-13 *ad hoc* for almonds by using an XDB-C8 column with a mobile phase gradient of dioxane-water-ACN (10+45+45 to 10+5+85). The results of validation experiments are shown in Table 53. The adaptation was also used for pecans.

Table 53. Recoveries of diflubenzuron from crop samples by method LAI 3-86-13 or its modifications. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike mg/kg	n	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference (type of trial) method version
original method								Duphar, 1984a, 1988b
pecan nuts	0.02	0.02 0.04 0.06 0.2 0.6 2	1 1 1 1 1 1	75 85 76 69 79 75	- - - - - -	- - - - - -	<0.006-0.006 (2)	Buisman and Verhaar, 1984 (validation report, DI-4415)
cotton seed	0.05	0.05 0.1 0.2	4 4 4	98 98 93	92-105 96-102 90-96	5.6 3.1 3.0	<0.05 (4)	Thiem, 1993, GLP (validation report, RP-93034)
cotton seed	0.05	0.05 0.5 2	4 2 2	99 91 92	92-105 90-91 92-93	5.6 - -	<0.05 (4)	Gaydosh, 1994a, GLP (residue trial, RP-93007)
soya bean seed	0.05	0.05 0.10	2 2	115 87	113-117 87-88	-	<0.015 (1)	Parker *et al.*, 1997, GLP (validation report, RP-96014)
soya bean seed	0.05	0.05 0.10	2 2	77 83	71-83 77-90	-	<0.05 (4)	Gaydosh, 2000f, GLP (residue trial, RP-96014)
modification A								CARDC-1248-DFB, Kane *et al.*, 1995
mushrooms	0.01	0.01 0.05 0.5	3 3 3	89 81 79	84-93 79-84 74-84	5.6 3.0 7.0	<0.003 (1)	Kane *et al.*, 1995, GLP (validation report, RP-96015)
mushrooms	0.01	0.1 1	16 16	80 83	62-97 72-110	8.7 12	<0.01-0.01 (16)	Gaydosh, 1999c, GLP (residue trial, RP-96015
soya bean forage	0.05	0.05 0.10	2 2	102 96	79-126 67-125	- -	<0.05-0.31 (4)	Gaydosh, 2000f, GLP (residue trial, RP-96014)
soya bean hay	0.05	0.05 0.10	2 2	172 96	93-250 93-96	- -	0.23-1.0 (4)	Gaydosh, 2000f, GLP (residue trial, RP-96014)
modification B								Rose, 1998a
mushrooms	0.01	0.01 0.2	2 2	77 94	72-82 92-97	-	0.004 (1)	Rose, 1998a, GLP (validation report, RP-97004)
mushrooms	0.01	0.1	32	97	79-112	8.0	<0.01 (16)	Gaydosh, 1998a, GLP (residue trial, RP-97004)
mushrooms (freshly spiked aged samples)	0.01	0.1	12	95	78-117	17	<0.003-0.005 (6)	Rose, 2000b, GLP (storage stability, RP-98024)
modification C								Rose 1999d
almonds, kernels	0.05	0.05 0.1 0.5	2 3 3	78 77 84	76-80 70-81 81-85	- 7.9 2.8	<0.05 (1)	Rose, 1999d (validation report, RP-98003)
almonds, kernels	0.05	0.05 0.1	2 4	82 90	80-84 81-99	- 10	<0.05 (5)	Gaydosh, 2000c, GLP (residue trial, RP-98003)

Sample	LOQ, mg/kg	Spike mg/kg	n	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference (type of trial) method version
pecans, kernels	0.05	0.05 0.1 0.5	2 4 4	73 80 77	70-76 79-81 70-82	- 1.2 6.6	<0.05 (5)	Gaydosh, 2000d, GLP, (residue trial, RP-99002)
almonds, hull	0.05	0.05 0.1 0.5	3 3 3	82 86 74	78-85 79-93 70-78	4.6 8.2 5.4	<0.05 (1)	Rose, 1999d (validation report, RP-98003)
almonds, hull	0.05	0.05 0.1 2.5	2 2 2	95 112 89	92-98 111-114 85-93	-	<0.05 (5)	Gaydosh, 2000c, GLP (residue trial, RP-98003)
pecans, hull	0.05	0.05 0.5 2.0	2 6 2	89 77 85	84-94 70-82 85-85	- 7.0 -	<0.05 (5)	Gaydosh, 2000d, GLP (residue trial, RP-99002)

Heekin and Rich (1987, non-GLP) described the determination of diflubenzuron in wheat and wheat products. Samples were extracted by cold shaking (16-20 hours) with ACN without prior grinding (wheat grain, bread crumbs) or with ethyl acetate (flour, bran offals). Extracts were dried with anhydrous sodium sulfate, evaporated to dryness and redissolved in mobile phase for HPLC (Spherisorb S5 ODS column, mobile phase ACN/water (60+40), 257 nm) with external standards in ACN. Bread was analysed on a Chromspher C-18 column.

The authors showed that diflubenzuron was fully extracted from wheat grain without prior grinding. Duplicate analyses of wheat with incurred residues (treated post-harvest at 2 g ai/tonne with 4 months' ambient storage) showed 1.36 (1.28, 1.43) mg/kg for a ground portion and 1.38 (1.32, 1.42) mg/kg for an unground portion.

A validation report was not available. The method was validated during trials with a limited number of recoveries. The results are shown in Table 54. The LOQ was not reported.

Table 54. Recoveries of diflubenzuron from wheat and wheat products by an Australian HPLC-UV method. Control samples showed no peaks.

Sample	Spike, mg/kg	No.	% REC mean	% REC range	%RSD$_r$	Reference
Wheat grain	2 4	2 2	97 94	95-98 93-95	-	Heekin and Rich, 1987, non-GLP (storage and processing trial, DI-5937)
First reduction flour	1	2	96	93-98	-	
Buehler flour	1	2	95	93-97	-	
Wholemeal four	1	2	95	95-95	-	
Bran	1	2	97	97-97	-	
Offals	1	2	102	98-105	-	
Wholemeal bread	1	3	90	87-95	4.8	
White bread	1	3	103	98-107	4.4	

Buisman and Dijkstra (1988, non-GLP) described "Method A" for the determination of diflubenzuron in cabbage. The extraction with ethyl acetate and clean-up (partitioning into ACN and purification on Florisil) was according to the GLC method LAI-3-86-6 (Duphar 1987c) for Brussels sprouts. The residue was dissolved in mobile phase for analysis by HPLC (C-8, RP-8 pre-column, mobile phase dioxane/water/ACN (10:45:45); 254 nm) with external standards.

There was no validation report. Analyses were validated in a trial on cabbage (Table 55).

Table 55. Recoveries of diflubenzuron from head cabbage by HPLC-UV method A.

LOQ, mg/kg	Spike, mg/kg	No.	% Rec	Controls mg/kg	Reference
0.6	1 2	1 1	93 98	<0.2 (4 samples)	Buisman and Dijkstra, 1988, non-GLP, (residue trial, DI-6955)

Method LAI 3-86-14, 26 April 1988 (Duphar, 1988e, non-GLP) described the determination of diflubenzuron in apples, pears and their processed commodities. Homogenized samples (50-100 g) were blended with anhydrous sodium sulfate, filter pulp and dichloromethane for apples, pears, dried apples and apple sauce or ethyl acetate for apple butter. Apple and pear juice (100 ml) were directly extracted with dichloromethane/ether (1+50) and filtered through anhydrous sodium sulfate. The filtered extract was evaporated to dryness, redissolved in dichloromethane/petroleum ether (3+25) and purified on a Florisil column (except apple butter). Apple butter was redissolved in ACN/water and partitioned into hexane. The final eluate or extract was evaporated to dryness and redissolved in mobile phase for analysis by HPLC (C-8 column; mobile phase dioxane-water-ACN 10+45+45; 254 nm) with external standards. The method was also used for mushrooms.

Pouwelse (1989, GLP) modified the method *ad hoc* for apples. The amount of filter pulp was decreased and the eluant for the Florisil column was changed.

There was no validation report. Analyses were validated in trials on apples and mushrooms. The results are shown in Table 56. The selectivity in the presence of DFBA, CPU and PCA was not verified for mushrooms.

Table 56. Recoveries of diflubenzuron by method LAI 3-86-14. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike mg/kg	n	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
original method								Duphar, 1988e
mushrooms	0.01	0.10	4	101	100-102	0.95	<0.003 (4)	Allan and Thus, 1996, non-GLP (residue trial, DI-9441),
modification								
apples	ns	-	-	-	-	-	0.004-0.028 (4)	Pouwelse, 1989, GLP (storage stability, DI-7240)

ns: not stated

Duphar (1988h, non-GLP) described the determination of diflubenzuron in tea by method RES 020. Homogenized samples (25 g) were refluxed for 30 min with ACN/water (85+15). The filtered extract was concentrated, washed with hexane, evaporated to dryness, redissolved in ACN, washed with hexane, evaporated to dryness and redissolved in dichloromethane/petroleum ether (3+25). The sample was purified on a Florisil column, the eluate evaporated to dryness and the residue redissolved in mobile phase. Diflubenzuron was determined by HPLC (C-8 with RP-8 guard column; mobile phase dioxane-water-ACN 10+45+45; 254 nm) with external standards. The method was not used as such but was modified by Massingill (1993, GLP) for fresh and dry grass. The final extract was filtered through a 0.2 μm filter before HPLC analysis.

The method was validated only during trials, with a limited number of recoveries. The results are shown in Table 57. The LOQ was 0.05 mg/kg.

Table 57. Recoveries of diflubenzuron from grass by a modification (Massingill, 1993) of method RES020. Numbers in parentheses are numbers of control samples.

Sample	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference

Sample	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
fresh grass	1 4	7 7	97 87	89-114 83-90	9.1 3.0	<0.05 (16)	Gaydosh, 1994b, GLP (residue trial, RP-91053)
fresh grass	1 4	3 3	90 80	85-93 79-80	4.8 0.7	<0.05-0.22 (3)	Gaydosh, 1993b, GLP (residue trial, RP-92001)
dry grass	1 4	2 2	86 78	86-86 77-79	- -	<0.05 (16)	Gaydosh, 1994b, GLP (residue trial, RP-91053)
dry grass	1 4	3 3	90 83	80-97 80-85	9.7 3.0	<0.05 (3)	Gaydosh, 1993b, GLP (residue trial, RP-92001)

Method RES060, Jul 1994 (Solvay Duphar, 1994b) was used for the determination of diflubenzuron in apples, apple juice and apple pomace. Homogenized samples (100 g) were mixed or ground with dichloromethane, anhydrous sodium sulfate and filter pulp. The filtered extract was evaporated to dryness and redissolved in methyl ethyl ketone/petroleum ether (2+25) and filtered. The filtrate was purified on a Florisil column; the eluate was evaporated to dryness, redissolved in mobile phase and filtered through a 0.45 µm filter. Diflubenzuron was determined by HPLC (C-18 column; mobile phase THF/ACN/water 10:40:50; 254 nm) with external standards in mobile phase.

Thus and Allan (1995, 1996, GLP) showed that calibration curves (two curves, each with 7 single standards (including blank) at 0.1-1.1 mg/l in mobile phase) were linear (r>0.999). Samples at 1 mg/kg were diluted 1:1 before injection. They modified the method *ad hoc* for apple juice by using filtered juice, by centrifugation instead of filtration, and by increasing the number of extractions to three. Analyses were validated only during trials (Table 58). The LOQ was 0.01 mg/kg.

Table 58. Recoveries of diflubenzuron from apple samples by method RES060. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike mg/kg	n	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference (type of trial) method version
original method								
apples	0.01	0.1 1	8 8	82 99	70-102 94-103	14 3.2	<0.003-0.011 (20)	Thus and Allan, 1995,1996, GLP (residue trial, DI-9320, processing, storage stability) RES060: July 1994.
apple pomace	0.01	0.1 1	4 4	77 98	67-88 95-102	13 2.9	<0.003-0.004 (2)	Thus and Allan, 1995,1996, GLP (residue trial, DI-9320, processing, storage stability) RES060: July 1994.
modification								Thus and Allan, 1995,1996
apple juice	0.01	0.1 1	4 4	99 102	97-103 97-108	2.7 4.6	<0.003 (2)	Thus and Allan, 1995,1996, GLP (residue trial, DI-9320, processing, storage stability)

Nowacki (1995, non-GLP) described a Polish method for the determination of diflubenzuron in apples. Samples (40 g) were blended with dichloromethane and filtered through anhydrous sodium sulfate. The dichloromethane extract was evaporated to dryness, the residue redissolved in acetone/petroleum ether (2+28) and purified by filtration through a solid reversed-phase filter (SRP-15). The filtrate was evaporated to dryness, redissolved in methanol and filtered again through SRP 15. Diflubenzuron was determined by HPLC (C-8, mobile phase 75% methanol in water, 254 nm) with external standards in methanol.

The method was validated only during a residue trial. The results are shown in Table 59.

Table 59. Recoveries of diflubenzuron from apples by Polish HPLC method.

LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Control, mg/kg	Reference

LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD_r	Control, mg/kg	Reference
0.0025	0.025 0.12	4 6	82 84	unknown unknown	7.3 8.6	<0.0025 (1 sample)	Nowacki, 1995, non-GLP (residue trial, DI-9620)

Method RES029, 6 February 1995 (Solvay Duphar, 1995, non-GLP) was applied to the determination of diflubenzuron in oranges and mandarins. Homogenized samples (50 g) were ground with dichloromethane and anhydrous sodium sulfate and filtered. The evaporated residue was dissolved in hexane, partitioned into ACN, the ACN evaporated and the residue redissolved in methyl ethyl ketone/petroleum ether (2+25), which was filtered through a 5 µm filter. The sample was purified as usual, redissolved in mobile phase and filtered through a 0.45 µm filter for HPLC (C-18 column; mobile phase THF/water/ACN 10+50+40; 254 nm) with external standards.

Van Dijk and Keetelaar-Janssen (1995, non-GLP) verified linearity with 6 single standards without substrate (range 0.11-11 mg/l) including blank; r=0.99999.

Limited validation data are shown in Table 60.

Table 60. Recoveries of diflubenzuron from citrus samples by method RES029.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	Controls mg/kg	Reference
mandarins	0.01	0.1 1	2 2	86 82	85-87 81-83	<0.01 (3)	Van Dijk and Keetelaar-Janssen, 1995, non-GLP (residue trial; DI-9331)
oranges	0.01	0.1 0.9	2 2	89 87	87-91 87-87	<0.01 (3)	

Method RES045, 23 January 1997 (Solvay Duphar, 1997, non-GLP) was used for oranges and lemons. Homogenized samples (50 g) were sonicated with water/ACN (1+4). After centrifugation, the supernatant layer was shaken with saturated NaCl. The ACN layer was washed with hexane, purified on a Florisil column, evaporated to dryness and the residue redissolved in mobile phase for HPLC (C-18 column; RP pre-column; mobile phase water-ACN 50+50; 254 nm) with external standards in mobile phase.

Allan *et al.* (1997, GLP) verified the linearity with 6 single standards (range 0.02-1.0 mg/l) including blank: r>0.99995 (3 curves).

Recoveries are shown in Table 61.

Table 61. Recoveries of diflubenzuron from citrus by method RES045. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD_r	Controls mg/kg	Reference
lemons	0.02	0.1 0.2 0.4 0.8 1.6 0.57(incurred)	1 1 1 1 1 5	106 94 93 93 95 -	- - - - - -	- - - - - 1.1	<0.006-<0.02 (17)	Allan *et al.*, 1997; GLP (residue trial, storage stability, DI-10942)
lemons	0.02	0.1 0.2 0.4 0.8 0.32(incurred)	4 4 4 4 5	52 62 60 63 -	46-60 54-67 48-68 57-67 -	14 9.5 15 6.7 3.7	<0.006-<0.02 (18)	Allan and Thus, 1999, GLP (validation, storage stability, DI-11424)

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
oranges	0.02	0.1	1	94	-	-	<0.006-<0.02 (17)	Allan *et al.*, 1997; GLP (residue trial, storage stability, DI-10942)
		0.2	1	92	-	-		
		0.4	1	85	-	-		
		0.8	1	89	-	-		
		1.6	1	88	-	-		
		0.64(incurred)	5	-	-	5.4		
oranges	0.02	0.1	4	76	70-86	8.9	<0.006-0.027 (18)	Allan and Thus, 1999, GLP (validation, storage stability, DI-11424)
		0.2	4	74	72-74	13		
		0.4	4	71	59-79	12		
		0.8	4	74	67-78	6.9		
		1.7	4	73	71-74	2.0		
		2.1	4	71	60-77	11		
		0.34(incurred)	5	-	-	12		

Method RES026, 14 December 1988 (Duphar, 1988i, non-GLP) was applied to the determination of diflubenzuron in soil. Diflubenzuron was extracted from 50 g soil by refluxing in water ACN (3+17) for 30 min. After cooling and filtration, part of the extract was concentrated and diflubenzuron was partitioned into hexane. After evaporation to dryness, the residue was redissolved in dichloromethane/petroleum ether (3+25) and cleaned up on a Florisil column. The eluate was evaporated to dryness and the residue redissolved in mobile phase (dioxane/ACN/water, 10+45+45) and analysed by HPLC (C-8 column with C-8 pre-column; UV 254 nm) with external standards (not matrix-matched).

Kramer (1990, 1991, 1992, GLP) modified the method *ad hoc* by changing the volume of extract taken for partitioning against hexane, the elution volumes in the clean-up step, the injection volume for HPLC and the standards used for calibration.

Validation reports (56635/34/90 and 56635/35/90) were not available to the reviewer. Validation data from other sources are shown in Table 62.

Table 62. Recoveries of diflubenzuron from soil by method RES026. Numbers in parentheses are numbers of samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference (type of trial)
Original method RES026								Duphar, 1988i
sandy loam soil	0.01	0.01	14	101	85-121	13	<0.01-0.01 (78)	Ver Hey, 1991a, GLP (field dissipation study)
		0.02	21	93	71-120	15		
		0.05	16	88	69-104	14		
		0.10	13	93	67-134	18		
		0.15	2	80	74-86	-		
		0.20	4	82	73-90	8.7		
		0.50	6	98	80-110	12		
		1.0	2	87	81-93	-		
		2.0	1	89	-	-		
sandy loam soil (freshly spiked aged samples)	0.01	0.5	5	89	76-104	12	<0.01 (23)	Ver Hey, 1991a, GLP (storage stability)
silty loam soil	0.01	0.01	15	104	75-136	18	<0.01-0.03 (70)	Ver Hey, 1991b, GLP (field dissipation study)
		0.02	16	94	64-129	19		
		0.05	8	93	86-102	5.7		
		0.1	16	91	73-124	15		
		0.2	1	91	-	-		
		0.5	9	93	79-101	8.0		
		1	3	97	93-105	7.2		
		2	2	100	99-101	-		
silty loam soil (freshly spiked	0.01	0.5	4	75	70-80	7	<0.01 (16)	Ver Hey, 1991b, GLP (storage stability)

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference (type of trial)
aged samples)								
Ad hoc modification								Kramer, 1990, 1991, 1992
loam soil	0.01	0.01	2	87	75-99	-	<0.01 (2)	Kramer, 1990, GLP (validation)
		0.1	2	81	79-84	-		
		1.0	2	105	93-117	-		
loam soil	0.01	0.02	1	102	-	-	<0.01 (17)	Kramer, 1990, GLP (field dissipation study)
		0.04	1	87	-	-		
		0.05	1	127	-	-		
		0.1	2	82	91-84	-		
		0.2	1	72	-	-		
		0.4	4	95	76-110	15		
		0.5	2	108	105-111	-		
		0.6	2	93	93-93	-		
		2	2	110	107-114	-		
		5	2	98	98-98	-		
loam soil (freshly spiked aged samples)	0.01	0.5	10	102	83-114	10	<0.01 (9)	Kramer, 1990, GLP (storage stability)
sandy soil	0.01	0.01	2	123	123-123	-	<0.01 (2)	Kramer, 1991, GLP (validation)
		0.1	2	90	83-98	-		
		1.0	2	94	94-94	-		
sandy soil	0.01	0.1	8	106	97-113	6.2	interference peak, conc not stated	Kramer, 1991, GLP (field dissipation study)
		0.2	2	116	114-118	-		
sandy soil (freshly spiked aged samples)	0.01	0.02	1	104	-	-	<0.01-0.031 (8)	Kramer, 1991, GLP (storage stability)
		0.1	1	110	-	-		
		0.2	2	82	80-84	-		
		0.5	5	101	95-113	7.0		
silt loam	0.01	0.01	8	105	98-118	6.2	<0.01 (4)	Kramer, 1992, GLP (validation)
		0.03	2	92	90-93	-		
		0.05	2	99	95-104	-		
		0.1	2	102	97-108	-		
		0.3	2	98	91-106	-		
silt loam	0.01	0.01	14	89	75-119	14	<0.01 (39)	Kramer, 1992, GLP (field dissipation study)
		0.1	15	100	75-116	12		
silt loam (freshly spiked aged samples)	0.01	0.5	5	79	72-95	13	<0.01 (5)	Kramer, 1992, GLP (storage stability)

Animal substrates

Corley *et al.* (1974, non-GLP) described and validated an HPLC-UV method for the determination of diflubenzuron in milk. Whole milk samples (100 g) were blended with anhydrous sodium sulfate and ethyl acetate. The extract was evaporated to dryness, the residue redissolved in ACN, and lipids removed by washing with hexane. The remaining ACN layer was evaporated to dryness and the residue redissolved in ethanol. Diflubenzuron was determined by HPLC-UV (Permaphase ODS, mobile phase methanol/water (1+1); 254 nm) with external standards in ethanol. The detector response was linear (r = 0.999 in the range 10-100 ng diflubenzuron, corresponding to 0.1-1 mg/kg). The metabolites PCA and DFBAM were recovered from fortified milk and were well separated from diflubenzuron by the chromatographic system (retention time relative to diflubenzuron 0.65 and 2.8 respectively).

Miller *et al.* (1976a, non-GLP) modified the method for milk, tissues and manure. Milk samples were 200–800 g. The cream was allowed to separate, siphoned off and extracted separately from the skimmed milk, which was extracted by the regular procedure. Ethyl acetate extracts from cream and milk were then combined into one sample. Tissue and manure samples (10-50 g manure, 50 g tissues) were mixed with anhydrous sodium sulfate and extracted by blending with ethyl acetate. Ethyl acetate extracts were analysed as for milk. The detector wavelength was changed to 220 nm.

Miller *et al.* (1976b, 1979, non-GLP) reported LOQs of 0.1 mg/kg in the fat, liver, muscle and eggs of chickens, and 0.02 mg/kg in the kidney, liver, muscle and fat of calves.

Validation are shown in Table 63. The selectivity in the presence of DFBA, CPU and PCAA was not verified.

Table 63. Recoveries of diflubenzuron from milk and tissues by HPLC-UV method of Corley *et al.*, 1974.

Sample	LOQ, mg/kg	Spike mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
milk	0.1	0.1 0.5 1	6 2 1	108 96 103	unknown 87-104	6.5	no peaks detected (1 sample)	Corley *et al.*, 1974, non-GLP (validation, DI-1576)
cow fat	0.1	0.1, 0.5, 1.0	?	93	unknown		?	Miller *et al.*, 1976a, non-GLP modified method
cow muscle	0.1	0.1, 0.5	?	94	unknown		?	
cow manure	0.1	0.1, 0.5, 1.0	?	94	unknown		?	

Thompson Hayward method AM-3 was used for the determination of diflubenzuron in tissues and milk in the cow metabolism study described earlier (Smith and Merricks, 1976a). The original method description was not available. Tissues were blended with ACN and milk with ethyl acetate. The mixtures were filtered, Celite Hyflo Supercel was added and the extract evaporated to dryness. Clean-up was on a Celite partition column followed by a mixed Florisil-alumina-silica gel column. The eluates were analysed on a Micro-Porasil column with detection at 254 nm.

The limited validation data are shown in Table 64.

Table 64. Recoveries of diflubenzuron from sheep kidneys by method AM-3.

LOQ, mg/kg	Spike, mg/kg	No.	% Rec	Controls mg/kg	Reference
0.05	0.05 0.1	1 1	70 85	<0.05 (2 samples)	Escobar, 1978, non-GLP (feeding study, DI-6840)

Smith and Merricks (1976a,b, non-GLP) compared method AM-3 with radiographic TLC quantification of radiolabelled incurred residues in tissues and eggs from a chicken metabolism study and liver from a cow metabolism study (Table 65).

The results indicate doubtful precision of the method.

Table 65. Determination of diflubenzuron by method AM-3 and by ^{14}C-TLC.

Sample	Total ^{14}C, mg/kg diflubenzuron equivalents	^{14}C-TLC analysis mg/kg parent	Method AM-3, mg/kg parent	Ratio AM-3/^{14}C	Reference
chicken fat	0.27	0.27	0.21	0.77	Smith and Merricks, 1976b
chicken muscle	0.043	0.029	<0.05	-	
chicken liver	0.21	0.039	0.16	4.1	
chicken kidney	0.13	0.030	<0.05	-	
chicken eggs	0.15	0.10	0.14	1.4	
cow liver	6.0	0.11	0.10	0.91	Smith and Merricks, 1976a

Di Prima (1976a, non-GLP) examined the specificity of method AM-3 by analysis of standard solutions of 87 of the 96 compounds having a tolerance in the USA in animal tissues and fish. The relative retention times of all of them were sufficiently different from diflubenzuron in one or two mobile phases; 9 compounds were not available and could not be tested. One compound, propachlor, had a relative retention time close to diflubenzuron with two mobile phases: 1.04 with dichloromethane/methanol (500+1) and 1.06 with iso-octane/isopropyl alcohol (96+4). When propachlor was carried through the whole analytical procedure (extraction, clean-up and analysis) however it was not detected.

Method LAI 3-86-17, 17 July 1984 (Duphar, 1984b, non-GLP) was used for the determination of diflubenzuron in eggs. Samples were blended with ACN and filtered. The filtrates were extracted with hexane after addition of sodium chloride and the extracts evaporated to dryness. The residues were dissolved in mobile phase for HPLC (C-8 column, dioxane/ACN/water (10+45+45); 254 nm).

The method was validated during feeding and storage stability trials, with the results shown in Table 66.

Table 66. Recoveries of diflubenzuron from eggs by method LAI 3-86-17. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
yolks	0.04	0.04 0.08 0.12 0.40 0.80	2 1 1 1 1	78 89 71 78 81	74-82 - - - -	- - - - -	<0.012 (7)	De Boer, 1993, non-GLP (feeding study, DI-5402)
yolks (freshly spiked aged samples)	0.05	0.05 0.5	4 4	66 67	62-74 61-73	7.9 9.9	<0.015 (4)	Buisman and Verhaar, 1987a, non-GLP (storage stability, DI-6206)
whites	0.04	0.04 0.08	2 2	80 71	79-82 67-75	- -	<0.012 (4)	De Boer, 1993, non-GLP (feeding study, DI-5402)
whites (freshly spiked aged samples)	0.05	0.05 0.1	4 4	76 76	72-78 70-83	3.9 7.1	<0.015 (4)	Buisman and Verhaar, 1987a, non-GLP (storage stability, DI-6206)

In method M-1590, 10 Oct 1985 (Kim and Manuel, 1985a, non-GLP) homogenized animal tissues were blended with ethyl acetate and anhydrous sodium sulfate. Fat was dissolved in ethyl acetate at 60-65°C. The filtrates were evaporated to dryness and the residues dissolved in hexane, partitioned into ACN, purified on a Florisil column, and redissolved in ACN for HPLC (Zorbax C-18 column; mobile phase ACN/water (60+40); 254 nm) with external standards in ACN.

Validation data are shown in Table 67. The reported LOQ was 0.05 mg/kg.

Kim and Manuel (1985b, non-GLP) observed that matrix interferences developed in liver samples stored at –20°C and concluded that the method was not suitable for liver samples stored frozen for more than 3 days (results were not reported).

Table 67. Recoveries of diflubenzuron from cattle tissues by method M-1590. Numbers in parentheses are numbers of control samples.

Sample	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
liver	0.05 0.1	3 2	88 88	80-97 80-96	9.9 -	<0.015 (3)	Kim and Manuel, 1985b, non-GLP, (validation, DI-8970)
liver	-	-	-	-	-	<0.015 (3)	Romano, 1985, non-GLP (feeding study, DI-8970)
muscle	0.05 0.1	4 2	90 84	75-101 83-84	12 -	<0.015 (2)	Kim and Manuel, 1985b, non-GLP (validation, DI-8970)
muscle	-	-	-	-	-	<0.015 (3)	Romano, 1985, non-GLP

Sample	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD_r	Controls mg/kg	Reference
							(feeding study, DI-8970)
kidney	0.05 0.1	3 2	80 94	78-82 87-100	2.6 -	<0.015 (2)	Kim and Manuel, 1985b, non-GLP, (validation, DI-8970)
kidney	-	-	-	-	-	<0.015 (3)	Romano, 1985, non-GLP (feeding study, DI-8970)
fat	0.05 0.1	3 2	96 88	83-109 87-88	14 -	<0.015 (3)	Kim and Manuel, 1985b, non-GLP, (validation, DI-8970)
fat	-		-		-	<0.015 (3)	Romano, 1985, non-GLP (feeding study, DI-8970)

Method M-1593, 18 November 1985 (Kim, 1985a, non-GLP) was developed for diflubenzuron in milk. Milk solids were precipitated by the addition of acetone. After filtration and evaporation of the acetone, the aqueous phase was extracted with hexane and the analysis completed as in M1590.

Validation data are shown in Table 68. The selectivity in the presence of DFBA, CPU, PCA and PCAA was not verified.

Table 68. Recoveries of diflubenzuron from cow milk by method M-1593. Numbers in parentheses are numbers of control samples.

LOQ, mg/kg	Spike mg/kg	No.	% Rec mean.	% Rec range	% RSD_r	Controls mg/kg	Reference
0.01	0.01 0.02 0.05	10 3 3	90 90 87	81-99 85-97 78-98	6.2 7.3 12	<0.003 (5)	Kim, 1985b, non-GLP (validation, DI-8969)
0.01	-	-	-	-	-	<0.003 (15)	Garces, 1985, non-GLP (feeding study, DI-8969)

HPLC method C, 20 March 1991 (Southworth, 1991) was used for the determination of diflubenzuron in liver and kidney. Diflubenzuron was extracted from the chopped sample (10 g) with ethyl acetate and anhydrous sodium sulfate. The extract was evaporated to dryness and partitioned between hexane and ACN (1+2). The ACN phase was dried and redissolved in dichloromethane/hexane (1+2). Final clean-up was by adsorption chromatography on silica gel. The eluate was evaporated to dryness and the residue redissolved in ACN for HPLC (C-8 column; mobile phase ACN/water (1+1); 254 nm) with external matrix-matched standards. The author reported verification results obtained by analysis of samples from an animal-treatment study (DI-9465), but the original data were not shown. The reported LOQ was 0.03 mg/kg in both liver and kidney. The RSD was 2% at 0.064 mg/kg with recoveries of 78%-82% at 0.05 mg/kg (matrix and number of recoveries not reported). The analytical response was linear in the range 0.03-5.0 mg/kg.

De Boer (1993, non-GLP) described an HPLC method for diflubenzuron in animal tissues. The extraction was based on the GC-ECD method LAI 3-86-18 (24 October 1985) and the determination on the HPLC method LAI 3-86-17 (17 July 1984). Homogenized tissues (25 g) were blended with ethyl acetate, anhydrous sodium sulfate and filter pulp. Fat (25 g) was dissolved in ethyl acetate by heating (temperature not reported). The filtrate was evaporated to dryness and the residue dissolved in hexane, partitioned into ACN, purified on a Florisil column, and transferred to mobile phase for HPLC (C-8 column, mobile phase dioxane/water/ACN, 10+45+45; 254 nm).

The limited validation data are shown in Table 69.

Table 69. Recoveries of diflubenzuron from chicken tissues by HPLC. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec	Controls mg/kg	Reference
fat	0.04	0.04 0.4 4 40	1 1 1 1	95 89 91 93	<0.012 (2)	De Boer, 1993, non-GLP (feeding study, DI-5402)
liver	0.04	0.04 0.08 0.2 0.6 1	1 1 1 1 1	59 83 92 92 89	<0.012 (2)	
kidney	0.04	0.04 0.08 0.4 1	1 1 1 1	90 79 92 93	<0.012 (2)	
muscle	0.04	0.04 0.08 0.2 0.6	1 1 1 1	90 88 93 92	<0.012 (2)	

In method STM CR59, 25 September 1997 (Analchem, 1997, non-GLP for the determination of diflubenzuron in ovine tissues, chopped liver, kidney or muscle (10 g) is blended with anhydrous sodium sulfate and ethyl acetate and fat with ACN. The fat extract is washed with hexane. The extracts are evaporated to dryness and the residues redissolved in hexane, partitioned into ACN, and the ACN evaporated. Liver and kidney residues are dissolved in cyclohexane/ethyl acetate (1+1) and cleaned up by GPC (Envirogel). All residues are redissolved in dichloromethane/hexane (1+2). Final clean-up is by adsorption chromatography on silica gel. The eluate is evaporated and the residue redissolved in mobile phase for HPLC (Spherisorb ODS column; mobile phase ACN/water (1+1); 254 nm). The linearity of the detector response was verified (4 single points) in the range 0.05-0.52 mg/l: r>0.9999 (Shepherd, 1998a,b).

Validation data are shown in Table 70.

Table 70. Recoveries of diflubenzuron from ovine tissues by method STM CR59. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
liver	0.02	0.02 0.05	5 1	86 88	63-103 -	19 -	<0.01 (1)	Shepherd, 1998a, GLP (animal treatment, DI-11404)
kidney	0.02	0.02 0.05 0.2	5 1 1	113 69 67	83-129 - -	19 - -	<0.01 (1)	
muscle	0.02	0.02 0.05 0.2	5 1 1	89 92 117	74-108 - -	14 - -	<0.006(1)	
fat	0.02	0.02 0.05 0.2	16 1 5	97 102 101	80-122 - 90-111	16 - 11	<0.006 (2)	
fat	0.02	0.02 0.2	5 5	82 77	62-89 64-84	14 10	<0.006 (3)	Shepherd, 1998b, GLP (animal treatment, DI-11403)

Timmerman *et al.* (1992c, non-GLP) described HPLC method D for diflubenzuron, CPU and PCAA in milk and liver. Liver samples were homogenized with skimmed milk (1:1; w/w). Milk samples and liver homogenates were spiked with [^{14}C]diflubenzuron and [^{14}C]CPU as internal standards and extracted with dilute ammonia. The ammonia extracts of liver were washed with pentane which extracts PCA (Timmerman *et al.*, 1992b). The ammonia extracts of milk and liver were extracted with diethyl ether, and the ether extracts washed successively with 1 M HCl to remove proteins and fatty acids, 4 g/l sodium sulfide (liver) to remove metal complexes or saturated decyltrimethylammonium bromide (milk) to degrade fatty acid micelles, and 1 M NaOH to remove remaining proteins and fatty acids. The ether was evaporated and the solvent changed to mobile phase for HPLC on an RP-8 column. The mobile phase was water/ACN/dioxane 50+40+10 for diflubenzuron and 70+25+5 for CPU and PCAA. Quantification was based on matrix-matched standards, which were extracted in the same way as the samples.

The extraction efficiency and repeatability for the internal standard [^{14}C]diflubenzuron (250 ng/g) was 27% ± 13% RSD from milk and 47% ± 6.2% from liver homogenate and of [^{14}C]CPU (250 ng/g) 10% ± 14% from milk and 34% ± 3.6% from liver homogenate. It was assumed that the extraction efficiency of PCAA was similar to that of CPU. The reported LOQ was 0.02 mg/kg for CPU in liver, 0.03 mg/kg for diflubenzuron, CPU and PCAA in milk, and 0.05 mg/kg for diflubenzuron and PCAA in liver. Control samples were not analysed. Recoveries from fortified milk and liver homogenates are shown in Table 71. Different concentrations of the internal standards in milk were used for calibration (150 ng/g CPU and 175 ng/g diflubenzuron) and for recoveries (250 ng/g CPU and diflubenzuron).

Comments. The principles of the method are not clear and interpretation of the data is therefore not possible. It is not clear why skimmed milk is used to homogenize the liver samples, why diflubenzuron does not partition into pentane, or why CPU and PCAA are not removed from the diethyl ether by washing in 1 M HCl. Finally it is not clear how the internal standards were used: were they added to separate aliquots of the sample, how were the UV absorbance data and radioactivity counts correlated for the determination of the real recovery and did the ^{12}C component not contribute to the recovery of the analytes?

Table 71. Recoveries of diflubenzuron, CPU, PCA and PCAA from goat liver and milk spiked with mixtures.

	Com-pound	Spike, mg/kg	Recovery from milk				Spike, mg/kg	Recovery from liver homogenate			
			No.	% Rec mean	% Rec range	% RSD_r		No.	% Rec mean	% Rec range	% RSD_r
mix 1	diflubenzuron	0.03	3	93	87-103	9	0.02	3[1]	127	50-190	56
	CPU	0.03	3	84	67-117	33	0.02	2[2]	160	155-165	-
	PCA	-	-	-	-	-	0.002	-	na	-	-
	PCAA	0.03	3	117	93-137	19	0.02	2[2]	110	45-175	-
mix 2	diflubenzuron	0.10	3	91	67-113	25	0.05	4	89	66-106	19
	CPU	0.10	3	94	63-113	29	0.05	3[1]	134	122-146	9
	PCA	-	-	-	-	-	0.005	-	na	-	-
	PCAA	0.10	3	105	93-117	11	0.05	3[1]	88	66-102	22
mix 3	diflubenzuron	0.18	5	83	71-96	11	0.20	-	na	-	-
	CPU	0.18	3	97	67-137	37	0.20	3[1]	112	92-142	24
	PCA	-	-	-	-	-	0.02	-	na	-	-
	PCAA	0.18	3	115	96-149	25	0.20	2[2]	113	89-136	-

[1] one run discarded (outlier or no signal, value not stated)

[2] two runs discarded (outliers or no signal, value not stated)

na: not analysed

LC-MS methods

In method CLE 808/185-04R, 11 November 1999 (Covance, 1999, non-GLP) for the determination of diflubenzuron in ovine kidney, liver, fat and muscle, homogenized samples were extracted with ACN, concentrated, and cleaned up on an SPE cartridge, and the evaporated residues were redissolved in water/ methanol/formic acid (60+40+0.5). Diflubenzuron was determined by LC-MS-MS (Hypersil

BDS C-18 column, mobile phase water/methanol/acetic acid (65+35+0.1); APCI positive ion mode) with external standards in mobile phase. Ions were monitored at m/z = 311 and 158 (MRM mode).

Batten, 2000, GLP) verified that the response was linear over the range 2-100 µg/l (10 point calibration, r>0.99, weighted linear regression). The results are shown in Table 72.

Comment. A single MS-MS transition may provide good selectivity but it does not provide complete specificity in all cases. Because the authors did not provide a rationalisation for the choice of m/z=158 as the only daughter ion and the differences between measured and expected ratio's between m/z 311 and 158 were not reported, the adequacy of the specificity cannot be evaluated.

Table 72. Recoveries of diflubenzuron from ovine tissues by method CLE 808/185-04R. Numbers in parentheses are numbers of samples.

Sample	LOQ, mg/kg	Spike mg/kg	n	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
liver	0.05	0.05 0.2	1 5	80 76	- 72-81	- 4.4	<0.015 (4)	Batten, 2000, GLP (animal treatment, DI-11657)
kidney	0.05	0.05 0.2	3 3	80 83	78-81 80-86	2.0 3.6	<0.015 (4)	
muscle	0.05	0.2	5	75	70-79	4.7	<0.015 (4)	
fat	0.05	0.05 0.2 0.4	1 3 2	81 67 72	- 54-93 63-81	- 34 (3) -	<0.015 (4)	

LC-MS method H for CPU. Willard (1998) described the determination of CPU in aquatic organisms (the original description of the method was not available).

Rose (1999d,f, GLP) modified the method for almonds and pecans. Homogenized samples were extracted twice with ACN, using a tissumiser for the first extraction and a sonicator for the second. The centrifuged or filtered extract was partitioned twice with hexane, concentrated and partitioned twice with hexane. The remaining ACN phase was evaporated to dryness, and the residue dissolved in ACN/water (1+4) and purified on a C-18 SPE cartridge. The eluate was partitioned twice with hexane, and the solvent changed to diethyl ether/hexane (1+19) for clean-up on a silica SPE cartridge. The eluate was evaporated to dryness and the residue dissolved in ACN/water (1+1). CPU was determined by HPLC-MS (C-18 column, gradient elution ACN/water/0.01% trifluoroacetic acid to ACN, APCI), with external standards in ACN/water (1+1).

Rose (1999c, 2000a, GLP) modified the method further for peaches and plums by introducing a water partition step to remove sugars. The ACN extract was evaporated to dryness and the residue dissolved in water (for peaches) or ethyl acetate (for plums) and partitioned between water and ethyl acetate. The ethyl acetate extracts were transferred to ACN/water (1+4) and purified on a C-18 SPE cartridge.

Validation data are shown in Table 73.

Table 73. Recoveries of CPU from crop samples by a modification of HPLC-MS method H. Numbers in parentheses are numbers of samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
modification A								Rose, 1999d,f
almond, kernels	0.005	0.005 0.01	3 3	88 96	84-94 95-97	6.0 10	<0.0015 (1)	Rose, 1999d, GLP (validation, RP-98003)
almond, kernels	0.005	0.005	6	112	101-129	11	<0.005 (5)	Gaydosh, 2000c, GLP (residue trial, RP-98003)
almond, hull	0.01	0.01	3	97	85-110	13	<0.01 (1)	Rose, 1999d, GLP

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD_r	Controls mg/kg	Reference
								(validation, RP-98003)
almond, hull	0.01	0.01	6	97	80-130	21	<0.01 (5)	Gaydosh, 2000c, GLP (residue trial, RP-98003)
pecan, kernels	0.005	0.005 0.01	2 6	78 77	75-82 69-90	- 10	<0.005 (5)	Gaydosh, 2000d, GLP (residue trial, RP-99002)
pecan, hull	0.01	0.010	8	78	74-83	4.6	<0.01 (5)	Gaydosh, 2000d, GLP (residue trial, RP-99002)
modification B								Rose, 1999c
peach	0.01	0.01 0.015	3 2	106 84	104-108 77-92	1.9 -	<0.003 (2)	Rose, 1999c, GLP (validation, RP-98001)
peach	0.01	0.01	8	97	77-128	16	<0.01 (6)	Gaydosh, 2000a, GLP (residue trial, RP-98001)
modification C								Rose, 2000a
plums	0.005	0.005 0.01	3 3	113 103	106-124 96-118	8.5 12	<0.0015 (2)	Rose, 2000a, GLP (validation, RP-98002)
plums	0.005	0.005	8	92	67-138	24	<0.005 (5)	Gaydosh, 2000b, GLP (residue trial, RP-98002)

GC methods

GC methods for the determination of diflubenzuron in plant products, animal products or soil are based on hydrolysis of diflubenzuron followed by derivatization with HFBA and determination by GC-ECD or GC-MS. The principle of the method is shown in Figure 4. Any CPU or PCA in the sample will be determined as diflubenzuron, if not separated before the hydrolysis step. Both CPU and PCA are formed by hydrolysis of diflubenzuron, but both are derivatized to the same product. If the clean-up after hydrolysis of diflubenzuron is selective for either CPU or PCA one of the compounds could be lost and diflubenzuron underestimated. The selectivity of the method therefore depends entirely on selective clean-up before the hydrolysis of diflubenzuron.

GC methods for the determination of CPU involve extraction followed by clean-up, derivatization with HFBA and determination by GC-ECD. Any PCA present in the sample will be determined as CPU if not separated before derivatization.

GC methods for the determination of PCA in plant or animal products are based on extraction under hydrolysis conditions followed by clean-up, derivatization with HFBA and determination by GC-ECD or GC-ID-MS. When hydrolysis conditions are strong enough any diflubenzuron in the sample will be determined as PCA if not separated before hydrolysis, and even under milder conditions CPU will be determined as PCA if not separated before derivatization.

GC methods for DFBA in mushrooms and soil are based on extraction under hydrolysis conditions followed by clean-up, derivatization with pentafluorobenzyl bromide (PFBBr) and determination by GC-ECD or by derivatization with diazomethane and determination by GC-MS. When hydrolysis conditions are strong enough, any diflubenzuron in the sample will be determined as DFBA if not separated before hydrolysis.

The main problem with the GC methods based on hydrolysis to PCA and acylation with HFBA is that they will detect almost anything which contains the PCA moiety, not only diflubenzuron. In addition, many other compounds including natural products will be acylated and, because detection is primarily a function of the acylating agent, may be detected with comparable sensitivity.

In the following descriptions of GC methods, unless otherwise stated, quantification is by comparison with external standards without added substrate, derivatized *in situ.*

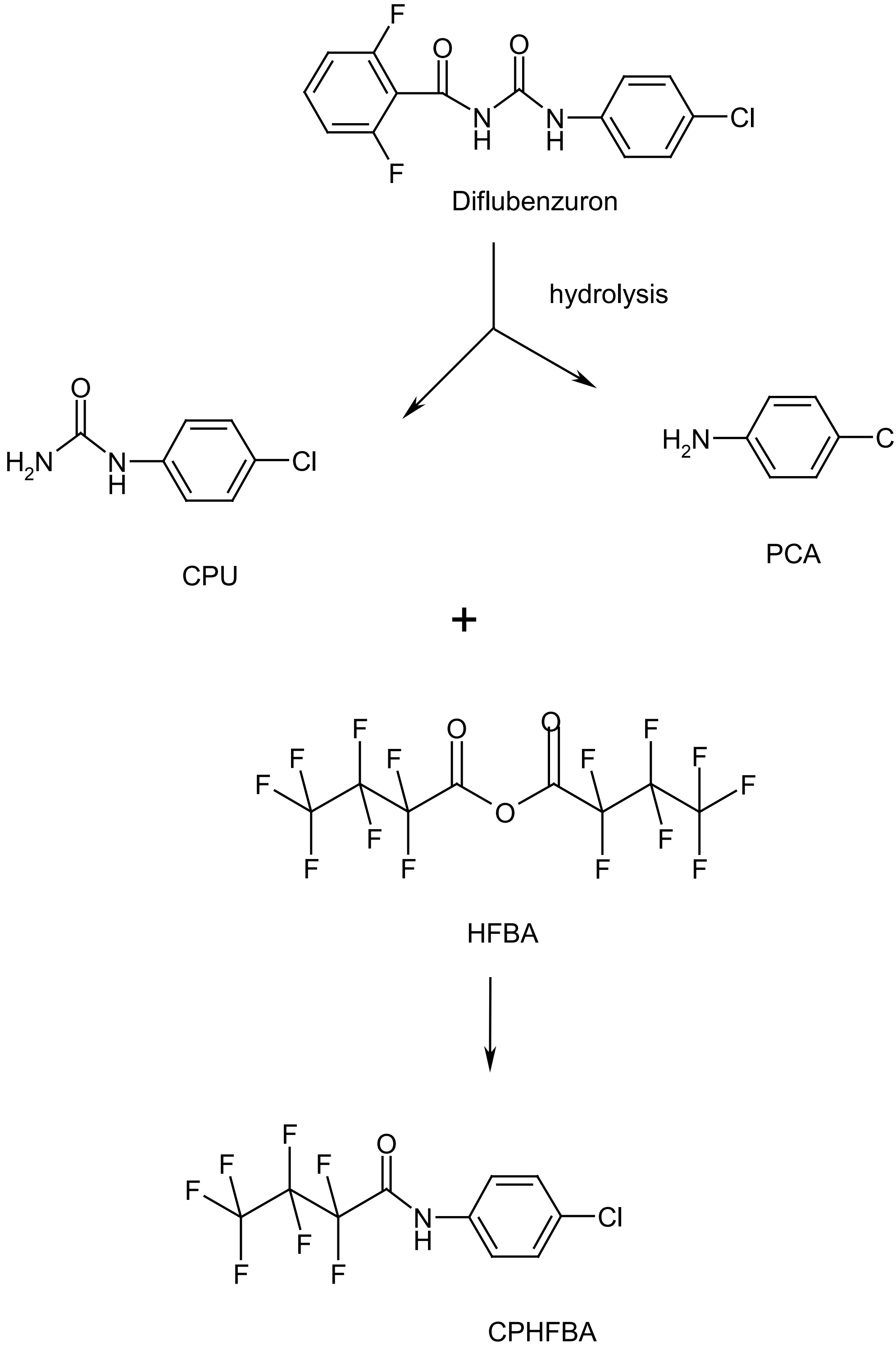

Figure 4. Hydrolysis of diflubenzuron and derivatization of CPU and/or PCA with HFBA in GC methods. Derivatization of CPU or PCA yields the same final product (CPHFBA) and the method cannot distinguish between the two metabolites.

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GC-ECD methods for diflubenzuron

Method LAI 3-86-6, 18 Jul 1978 (Duphar, 1978, non-GLP) was developed for the determination of diflubenzuron in apples and Brussels sprouts. Homogenized apples (100 g) or Brussels sprouts (50 g) were blended with anhydrous sodium sulfate, filter pulp, and dichloromethane or ethyl acetate respectively. The filtered extract from Brussels sprouts was evaporated to dryness, dissolved in hexane and partitioned into ACN. The filtered extract of apples or the ACN extract of Brussels sprouts was transferred to dichloromethane/petroleum ether (3+25), purified on a Florisil column and concentrated to dryness. The residue was resuspended in 85% phosphoric acid and refluxed for 30 min, when diflubenzuron residues were converted to PCA. The hydrolysates were washed with hexane, basified with NaOH (pH >12) and extracted with hexane. The PCA in the final hexane extract was derivatized with HFBA at room temperature and the product (CPHFBA) determined by GC-ECD on a Carbowax 20-M column. The method was also used for pears and oranges.

In 1987 (Duphar, 1987b,c, non-GLP, 8 September 1987, 23 November 1987) the method was extended to artichokes, which were extracted in the same way as Brussels sprouts.

In 1996 Morgenstern (1996a,d) modified the Duphar method for rice grain and straw and renamed it CARDC-1262-DFB (19 June, 1996). Samples (25 g) were treated as before but the GC column was changed to DB-1701.

Kane *et al.* (1997) modified the Duphar method for cotton seed and gintrash and named it CARDC-1264-DFB (6 Oct, 1997). Cotton seed and gintrash (25 g) were treated as before but a DB-17 or DB-1701 GC column was used.

Several *ad hoc* modifications were made by changing the amount of sample, the dichloromethane extraction volume, the filtration apparatus, the blending and shaking times, the elution volumes in the column chromatography, the GC columns (to DB-1701 or DB-17), temperature programmes or injection volumes, or by increasing the derivatization reagent, or adding extra washing steps after derivatization.

Validation data are shown in Table 74.

Table 74. Recoveries of diflubenzuron from crop samples by method LAI 3-86-6 or a modification. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference (type of trial) method version
original method								Duphar, 1978
Brussels sprouts	0.04	0.04	1	75	-	-	0.15-0.46 (2)	De Wilde, 1978e, non-GLP (residue trial, DI-1418)
		0.1	1	64	-	-		
		0.4	1	73	-	-		
		1	1	75	-	-		
		2	1	82	-	-		
Brussels sprouts	0.01	0.01	1	92	-	-	0.004 (2)	De Wilde, 1980, non-GLP (residue trial, DI-201
		0.05	1	80	-	-		
		0.07	1	84	-	-		
		0.1	1	76	-	-		
		0.7	1	79	-	-		
apple	0.01	0.1	1	65	-	-	<0.05 (4)	Duphar, 1985a, non-GLP (residue trial, DI-5054)
		1.0	1	87	-	-		
pear	0.01	0.05	1	66	-	-	<0.05 (4)	
		0.1	1	60	-	-		
		1	1	88	-	-		
orange	0.05	0.05	3	88	77-102	15	<0.05 (7)	Duphar, 1986b, non-GLP (residue trial, DI-5514)
		0.1	1	78	-	-		
		1	2	89	82-96	-		

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference (type of trial) method version
modification A								Duphar, 1986a, non-GLP (residue trial, DI-5295)
orange	0.05	1.0	1	119	-	-	<0.05 (1)	
modification B								Van Zijtveld *et al.*, 1989, non-GLP (residue trial, storage stability, DI-7492)
orange	0.01	0.5	2	86	85-86	-	<0.003-0.020 (14)	
grapefruit	0.05	-	-	-	-	-	<0.015 (10)	
modification C, CARDC-1264-DFB								Kane *et al.*, 1997
cotton seed	0.2	0.2	2	81	80-82	-	<0.006 (1)	Kane *et al.*, 1997, GLP (validation, RP-95017)
cotton seed	0.2	0.2 1 5	2 1 1	76 70 105	74-77 - -	- - -	<0.2 (2)	Gaydosh, 2000e, GLP (residue trial, RP-95017)
cotton gintrash	0.2	0.2	2	83	82-83	-	<0.006 (1)	Kane *et al.*, 1997, GLP (validation, RP-95017)
cotton gintrash	0.2	0.2 0.5 1 2 5	2 3 1 1 1	57 64 63 51 75	49-66 60-67 - - -	- 5.1 - - -	<0.2 (2)	Gaydosh, 2000e, GLP (residue trial, RP-95017)
modification D, CARDC-1262-DFB								Morgenstern, 1996a
rice grain	0.01	0.01 0.05 0.5	5 6 6	74 84 87	61-83 61-100 67-107	11 16 17	<0.003 (3)	Morgenstern, 1996a, GLP (validation report, RP-95016)
rice grain	0.01	0.01 0.05 0.5	3 3 3	76 96 83	61-99 85-116 75-94	26 19 12	<0.01 (4)	Gaydosh, 1996, GLP (residue trial, RP-95016
rice straw	0.01	0.01 0.025 0.05 0.5	12 6 12 6	80 85 77 61	49-120 65-107 58-99 46-83	26 20 17 27	<0.003-0.005 (6)	Morgenstern, 1996a, GLP (validation, RP-95016)
rice straw	0.01	0.01 0.025 0.05	1 1 1	122 98 99	- - -	- - -	<0.01 (4)	Gaydosh, 1996, GLP (residue trial, RP-95016
modification E								Nishioka and Rose, 1997a
rice grain	0.01	0.01 0.05 0.1	4 4 4	85 99 98	60-119 85-114 79-118	32 14 22	<0.003-0.008 (3)	Nishioka and Rose, 1997a, GLP (validation, RP-96032)
rice grain	0.01	0.03	9	101	78-132	20	<0.01 (7)	Gaydosh *et al.*, 1997a, GLP (residue trial, RP-96012)
rice grain (freshly spiked aged samples)	0.01	0.1	12	78	51-100	19	<0.003-0.011 (6)	Hathcock and Ruzo, 1998b, GLP (storage stability, RP-97026)
whole rice	0.01	0.03	2	81	81-81	-	<0.01 (2)	Gaydosh, 1998b, GLP (processing, RP-95028)
whole rice	0.01	0.03	2	119	115-123	-	<0.01 (2)	Gaydosh *et al.*, 1997b, GLP (processing, RP-96013)
white rice	0.01	0.03	2	80	76-83	-	<0.01 (2)	Gaydosh, 1998b, GLP (processing, RP-95028)
white rice	0.01	0.03	2	120	118-122	-	<0.01 (2)	Gaydosh *et al.*, 1997b, GLP (processing, RP-96013)
rice straw	0.01	0.01 0.05 0.1	4 4 4	109 109 109	98-116 105-114 107-113	7.6 3.4 2.5	<0.003 (2)	Nishioka and Rose, 1997a,GLP (validation, RP-96032)
rice straw	0.01	0.03	2	96	83-108	-	<0.01 (2)	Gaydosh, 1998b, GLP (processing, RP-95028)
rice straw	0.01	0.03	10	101	75-142	23	<0.01 (7)	Gaydosh *et al.*, 1997a, GLP (residue trial, RP-96012)
rice straw	0.01	1.0	2	82	82-83	-	0.01 (2)	Gaydosh *et al.*, 1997b, GLP (processing, RP-96013)
rice straw (freshly spiked aged samples)	0.01	0.1	12	82	40-110	24	<0.003-0.01 (6)	Hathcock and Ruzo, 1998b, GLP (storage stability, RP-97026)
rice hulls	0.05	0.05	4	98	87-111	10	0.0051-0.0090 (2)	Nishioka and Rose, 1997a,GLP

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference (type of trial) method version
		0.1	4	90	80-100	11		(validation, RP-96032)
rice hulls	0.05	0.03	2	70	67-74	-	<0.05 (2)	Gaydosh, 1998b, GLP (processing, RP-95028)
rice hulls	0.05	0.03	2	108	102-115	-	<0.05 (2)	Gaydosh *et al.*, 1997b, GLP (processing, RP-96013), Nishioka and Rose, 1997a
rice hulls (freshly spiked aged samples)	0.05	0.1	12	84	60-104	19	<0.003-0.01 (6)	Hathcock and Ruzo, 1998b, GLP (storage stability, RP-97026)
rice bran	0.01	0.01 0.05 0.1	4 4 4	102 96 79	79-117 89-103 74-84	16 7.6 6.0	<0.003 (2)	Nishioka and Rose, 1997a,GLP (validation, RP-96032)
rice bran	0.01	0.03	2	68	58-79	-	<0.01 (2)	Gaydosh, 1998b, GLP (processing, RP-95028)
rice bran	0.01	0.03	2	96	96-96	-	0.01 (2)	Gaydosh *et al.*, 1997b, GLP (processing, RP-96013)
rice bran (freshly spiked aged samples)	0.01	0.1	12	69	10-110	49	<0.003-0.004 (6)	Hathcock and Ruzo, 1998b, GLP (storage stability, RP-97026)
modification F								Rose, 1998c
rice grain	0.01	0.01	6	82	60-118	29	<0.01 (5)	Gaydosh and Puhl, 1998,GLP (residue trial, RP-98029)
rice straw	0.01	0.01	6	82	60-101	21	<0.01 (5)	Gaydosh and Puhl, 1998, GLP (residue trial, RP-98029)
modification G								Rose, 1998b
lettuce	0.01	0.01 0.05 0.1	2 2 2	124 96 92	116-132 93-100 91-92	- - -	<0.003 (2)	Rose, 1998b, GLP (validation, RP-95018)
lettuce	0.01	0.03	26	85	70-101	9.4	<0.003-0.006 (13)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
lettuce (freshly spiked aged samples)	0.01	0.1	12	77	42-118	27	<0.003-0.021 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
turnip tops	0.01	0.01 0.05 0.1	2 2 2	74 90 84	74-74 90-90 84-85	- - -	0.003 (2)	Rose, 1998b, GLP (validation, RP-95018)
turnip tops	0.01	0.03 0.06	22 2	98 97	72-119 97-97	18 -	<0.003-0.007 (12)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
turnip roots	0.01	0.01 0.05 0.1	2 2 2	103 81 80	102-103 81-82 80-80	- - -	<0.003 (2)	Rose, 1998b, GLP (validation, RP-95018)
turnip roots	0.01	0.03	24	99	70-119	13	<0.003-0.010 (12)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
turnip roots (freshly spiked aged samples)	0.01	0.1	12	82	60-108	14	<0.003-0.017 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
wheat forage	0.01	0.01 0.05 0.1	2 2 2	102 94 116	99-105 92-95 113-119	- - -	0.007 (2)	Rose, 1998b, GLP (validation, RP-95018)
wheat forage	0.01	0.03	18	100	72-118	14	0.005-0.012 (9)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
wheat grain	0.01	0.01 0.05 0.1	2 2 2	84 109 109	84-84 109-110 108-110	- - -	0.003-0.004 (2)	Rose, 1998b, GLP (validation, RP-95018)
wheat grain	0.01	0.03	20	94	73-116	16	<0.003-0.011 (10)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
wheat grain (freshly spiked aged samples)	0.01	0.1	12	90	54-122	27	<0.003-0.007 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
wheat hay	0.01	0.01 0.05 0.1	2 2 2	102 113 89	101-103 112-115 87-90	- - -	0.007 (2)	Rose, 1998b, GLP (validation, RP-95018)

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference (type of trial) method version
wheat hay	0.01	0.03	22	85	74-106	11	<0.003-0.011 (11)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
wheat hay (freshly spiked aged samples)	0.01	0.1	12	88	61-122	24	<0.003-0.007 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
wheat straw	0.01	0.01 0.05 0.1	2 2 2	66 84 95	64-67 79-89 91-98	- - -	0.004-0.005 (2)	Rose, 1998b, GLP (validation, RP-95018)
wheat straw	0.01	0.03	22	102	82-121	12	0.004-0.015 (11)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
modification H								Nishioka *et al.*, 1997
pear	0.05	0.05 0.5 2	1 2 2	74 81 72	- 75-87 67-78	- - -	<0.015 (1)	Nishioka *et al.*, 1997, GLP (validation, RP-96025)
pear	0.05	0.05	4	90	79-114	18	<0.05 (2)	Gaydosh and Rose, 1999, GLP (residue trial, RP-96025)
pear	0.05	0.05 0.3 1.2	12 2 2	73 78 81	62-82 75-81 73-89	7.3 - -	<0.05 (14)	Dorschner and Gaydosh, 2000, GLP (residue trial, IR-4)
pear (freshly spiked aged samples)	0.05	0.05	6	75	70-81	4.5	<0.05 (3)	Nishioka *et al.*, 1997, GLP (storage stability, RP-96025)
modification I								Rose, 1999a
peach	0.005	0.005 0.01 1	2 2 2	94 94 86	94-95 85-102 86-86	- - -	0.0027 (1)	Rose, 1999a, GLP (validation, RP-97008)
peach	0.005	1.0	12	86	70-101	14	<0.005 (6)	Gaydosh, 1999b, GLP (residue trial, RP-97008)
Modification J								Rose, 1999c
peaches	0.05	0.05 0.1	3 3	80 89	65-95 75-101	19 15	<0.015 (2)	Rose, 1999c, GLP (validation, RP-98001)
peaches	0.05	0.05 0.06 0.1	4 2 4	90 83 65	79-98 81-85 63-67	8.9 - 2.5	<0.05 (10)	Gaydosh, 2000a, GLP (residue trial, RP-98001)
Modification K								Rose, 2000a
plums	0.05	0.05 0.1	3 3	84 85	79-89 83-86	6.0 1.8	<0.015 (2)	Rose, 2000a, GLP (validation, RP-98002)
plums	0.05	0.05 0.1	6 2	78 89	70-87 87-91	7.7 -	<0.05 (5)	Gaydosh, 2000b, GLP (residue trial, RP-98002)
prunes	0.05	0.05 0.1	3 3	70 71	63-76 65-76	9.3 8.0	<0.015 (2)	Rose, 2000a, GLP (validation, RP-98002)
prunes	0.05	0.1	2	70	69-72	-	<0.015 (1)	Gaydosh, 2000b, GLP (residue trial, RP-98002)
Modification L								Rose, 1999b
pepper	0.05	0.05 0.5 1	6 6 6	80 96 87	66-85 87-98 82-92	10 5.9 5.7	<0.015-0.02 (4)	Rose, 1999b, GLP (validation, RP-97016)
pepper	0.05	0.2	10	111	82-133	14	<0.05 (9)	Gaydosh and Puhl, 1999, GLP (residue trial, RP-97016)
pepper (freshly spiked aged samples)	0.05	0.2 0.4	8 2	92 72	77-137 70-73	25 -	<0.015-0.047 (5)	Rose, 1999b, GLP (storage stability, RP-97016)

Duphar (1985a) compared method LAI 3-86-6 with HPLC method LAI 3-86-2 as a reference method. No bias was found in the range <0.05-0.9 mg/kg diflubenzuron (paired t-test) for 8 samples each of apples and pears (Table 75).

Although the t-test showed no difference between the methods, the agreement is sometimes quite poor if the samples are considered individually.

Table 75 Comparison between HPLC method LAI-3-86-2 and GC-ECD method LAI 3-86-6.

Apple[1]			Pear[1]		
HPLC-UV LAI 3-86-2	GC-ECD LAI 3-86-6	Difference	HPLC-UV LAI 3-86-2	GC-ECD LAI 3-86-6	Difference
0.1	0.2	-0.1	0.3	0.3	+0.0
0.2	0.3	-0.1	0.3	0.2	+0.1
0.2	0.3	-0.1	0.3	0.3	+0.0
0.2	0.3	-0.1	0.4	0.5	-0.1
0.4	0.4	+0.0	0.4	0.4	+0.0
0.7	0.4	+0.3	0.4	0.3	+0.1
0.9	0.5	+0.4	0.9	1.1	-0.2
0.9	0.5	+0.4	0.9	0.5	+0.4

[1] samples containing <0.05 mg/kg were omitted (3 apples and 4 pears)

Modification E for the determination of diflubenzuron in rice was proposed as an enforcement method. The structure of HFBA-derivatized PCA was confirmed by GC-MS (electron impact) at m/z 323 (parent) and the characteristic fragments m/z 126 and 154 (Nishioka and Rose, 1997a, GLP).

Jalali and Hiler (1997, GLP) compared modification E with HPLC and LSC in [^{14}C]diflubenzuron-treated rice plants from the metabolism study described by Walsh (1997). For the HPLC-LSC method the grain was extracted twice with methanol-water (1:1; 1% acetic acid) and once with ACN-1M HCl (1:1), and the straw twice with ACN, twice with the ACN-water and once with the ACN-HCl. After concentration of the extracts, diflubenzuron was determined by HPLC-LSC on a C-18 column with gradient elution and a fraction collector.

The results are shown in Table 76. The proposed method showed no bias (<1.0%) for incurred radioactive residues in the presence of CPU (0.14-0.25 mg/kg diflubenzuron equivalents) and PCA (0.002 mg/kg diflubenzuron equivalents). The diflubenzuron levels in the two studies were comparable: 0.33-0.34 mg/kg in the Jalali and Hiler study and 0.38 mg/kg in the original study indicating no storage instability during 12 months.

Independent laboratory validations were not available for rice, a confirmatory technique was not proposed and the method was not generally available (unpublished). Hydrolysis and derivatization efficiency for diflubenzuron were verified, indirectly, by the bias study.

Table 76. Comparison of diflubenzuron methods in ^{14}C-treated rice plants.

Sample	Treatment (kg ai/ha)	TRR mg/kg diflubenzuron equivalents (Walsh, 1997)	TRR mg/kg diflubenzuron equivalents (Jalali and Hiler, 1997)	Distribution in extracts (Walsh, 1997)	diflubenzuron (mg/kg) HPLC-LSC (Jalali and Hiler, 1997)	diflubenzuron (mg/kg) GC-ECD (Jalali and Hiler, 1997)
grain	1.7	0.66	0.64	diflubenzuron 0.3% CPU 22%; CPU conj 0.9% PCA 0.3%	0.0	0.0
straw	0.28	1.0	0.88	diflubenzuron 36%; CPU 26%; PCA not detected	0.33	0.34

In method RES013, 11 September 1983 (Duphar, 1983, non-GLP) for the determination of diflubenzuron in walnuts, homogenized samples (50 g) were blended with ethyl acetate. The filtered extract was concentrated and the remaining oily residue dissolved in hexane. Diflubenzuron was partitioned into ACN, which was washed with hexane, and transferred to dichloromethane/petroleum ether (3+25). The analysis was completed as in LA13-86-6.

Koch (1993, GLP) modified the method *ad hoc* for walnuts by using only part of the ethyl acetate extract to lower the amount of oil, hydrolysing for 20 instead of 30 min, introducing extra extraction steps, changing elution volumes in the column chromatography, increasing the derivatization reagent, and changing the calibration concentrations, the GC column packing and the injection volume.

Anthony and Brown (2000, GLP) modified the method for walnuts and renamed it Agrisearch DB/99/1. The initial ethyl acetate extract was partitioned into ACN, which was washed with hexane, evaporated to dryness and the residue dissolved in cyclohexane/ethyl acetate (50:50). This was purified by GPC (SX-3 Envirobeads). Eluates were concentrated to dryness, hydrolysed and derivatized as in the original method. The analytical column was fused silica with HP wax.

A validation report was not available. The limited validation data are shown in Table 77. The hydrolysis and derivatization efficiencies were not verified.

Table 77. Recoveries of diflubenzuron from walnuts by modifications of method RES013 or a modification. Numbers in parentheses are numbers of samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference (type of trial) method version, GLP
modification A								Koch, 1993
walnut, kernels	0.05	0.05 0.10	3 3	79 72	76-80 70-74	2.9 2.9	<0.05 (6)	Gaydosh, 1993a, GLP (residue trial, RP-88022)
modification B								(Agrisearch DB/99/1)
walnut, kernels	0.05	0.05 0.2	1 1	86 70	- -	- -	-	Anthony and Brown, 2000, GLP (residue trial, DI-11544)

Duphar (1990, non-GLP) described the determination of diflubenzuron in citrus. The original method was not available to the reviewer, and the following description may include modifications which could not be identified. Homogenized samples were blended with acetone. The filtered extract was concentrated, aqueous NaCl added, and diflubenzuron was partitioned into dichloromethane, transferred to hexane and partitioned into ACN, which was evaporated to dryness. The analysis then followed LAI3-86-6, except that a DB-1701 column was used.

Validation data are shown in Table 78. The hydrolysis and derivatization efficiency were not verified.

Table 78. Recoveries of diflubenzuron from citrus samples by the method of Duphar (1990). Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
lime	0.01	0.01 0.05 0.5	2 2 2	78 72 96	77-80 72-73 95-97	- - -	0.0052 (1)	Rose and McIntosh, 1998, GLP (validation, RP-96028)
lime	0.01	0.3	2	88	86-90	-	0.038, 0.046 (2)	Gaydosh, 1999a, GLP (residue trial, RP-96028)
lime (freshly spiked aged samples)	0.01	0.5	8	92	81-108	11	<0.003 (4)	Rose and McIntosh, 1998, GLP (storage stability, RP-96028)
lemon	0.01	0.3	2	110	109-112	-	<0.003 (2)	Gaydosh, 1999a, GLP (residue trial, RP-96028)

Method LAI 3-86-18 (Duphar, 1985b,1998c, non-GLP) determines diflubenzuron in animal tissues. Homogenized tissues were blended with ethyl acetate and anhydrous sodium sulfate, and fat dissolved in ethyl acetate by heating (temperature not reported). The filtrate was evaporated to dryness and the residue dissolved in hexane and partitioned into ACN. After purification on a Florisil column, the evaporated eluate was hydrolysed with concentrated phosphoric acid (reflux for 30 min). After clean-up with hexane, the aqueous phase was basified to pH >12 and the hydrolysis product (PCA) extracted with hexane. PCA was derivatized with HFBA and determined by GC-ECD on a Carbowax 20-M column.

A validation report was not available. The limited validation data are shown in Table 79. The hydrolysis and derivatization efficiency and the selectivity in the presence of CPU and PCA were not verified.

Table 79. Recoveries of diflubenzuron from pigs' liver and kidney by method LAI 3-86-18.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec	Controls mg/kg	Reference
liver	0.01	0.01 0.03 0.05 0.1	1 1 1 1	73 62 89 93	<0.003, 0.007	De Wilde and Buisman, 1986, non-GLP (metabolism study, DI-5592)
kidney	0.03	0.03 0.05 0.1	1 1 1	69 58 57	<0.009 (2)	

Kramer (1991, GLP) described a GC-ECD determination of diflubenzuron in soil (Method L). The method combines the sample clean-up of HPLC method RES026 (Duphar, 1988i) and the GC determination of method LAI 3-86-25 (Duphar, 1988k). Diflubenzuron was extracted from 50 g soil by refluxing in water/ACN (3+17) for 30 min. After cooling and filtration, part of the extract was concentrated and diflubenzuron partitioned into hexane. After evaporation to dryness, the residue was redissolved in dichloromethane/petroleum ether (3+25) and cleaned up on a Florisil column. Diflubenzuron in the eluate was hydrolysed with phosphoric acid for 30 min under reflux and washed with hexane. The pH was adjusted to 12 with NaOH and diflubenzuron partitioned into hexane. Part of the hexane extract was treated with HFBA and analysed by GC-ECD (packed Carbowax column).

Validation data are shown in Table 80. The hydrolysis and derivatization efficiency for diflubenzuron and the selectivity in the presence of CPU and PCA were not verified.

Table 80. Recoveries of diflubenzuron from sandy soil by method L. Numbers in parentheses are numbers of control samples.

LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD_r	Controls mg/kg	Reference
0.01	0.01 0.1 1	2 2 2	119 81 74	115-123 72-90 70-77	- - -	<0.01 (2)	Kramer, 1991, GLP (validation)
0.01	0.1 0.2 0.4	15 5 1	98 97 89	78-119 76-108 89-89	11 14 -	<0.01-0.098 (22)	Kramer, 1991, GLP (field dissipation study)

GC-ECD methods for diflubenzuron and/or metabolites

Thompson Hayward method AM-15A, 2 November 1976 (DiPrima, 1976b, non-GLP) is for the determination of diflubenzuron, CPU and PCA in cow tissues, poultry tissues, eggs, milk and fish. Homogenized samples (20 g) were blended with ethyl acetate for milk, ACN/ethyl acetate (1+1) for muscle, liver, kidney and eggs, hexane for fat, or methanol for fish. Hexane-fat solutions were partitioned with ACN. The filtered extracts and the ACN phase from fat were concentrated to near dryness, diluted with water and adjusted to pH <2 (solution A). Diflubenzuron was extracted from solution A with hexane and partitioned into ACN. The pH in solution A was adjusted to >10 and PCA was extracted with hexane/benzene (7+3) and dried with anhydrous sodium sulfate. Finally, CPU was extracted from the alkaline solution A with diethyl ether. The PCA extract was cleaned up on an alumina column, derivatized with HFBA for 3-5 h at 50-55°C and cleaned up on a Florisil column. The diflubenzuron and CPU extracts were cleaned up separately on Florisil columns, and both hydrolysed for 14 hours with 12-M HCl (reflux) to form PCA. The hydrolysate was washed with benzene, adjusted to pH >10 and the PCA extracted with benzene and derivatized with HFBA as before. Final eluates from the Florisil column were transferred to hexane for CPU and PCA determinations or iso-octane for diflubenzuron. Derivatized PCA (CPHFBA) from all three extracts was determined by GC-ECD (OV-17, Carbowax 20-M or OV-3 columns) with external CPHFBA standards (7 single standards, range 2-40 pg) in hexane.

Escobar (1978, non-GLP) modified the method by extracting sheep fat with methanol.

DiPrima (1976b, non-GLP) found that the average conversion of diflubenzuron and CPU standards to PCA was 96% ± 5.5% over a range of 0.2-1000 µg. The efficiency of derivatization of PCA standards with HFBA was 103% ± 8.9% over the same range. A minimum of 2 hours is needed for complete derivatization.

Results of validation and feeding studies are shown in Table 80. The selectivity for diflubenzuron, CPU and PCA in each other's presence was indirectly verified because satisfactory results were obtained from the three separate extracts. The LOQ was 0.05 mg/kg for all analytes in all samples from tissues, milk and eggs.

Table 81. Recoveries of diflubenzuron, CPU and PCA from tissues, milk and eggs by method AM-15A. Numbers in parentheses are numbers of control samples.

Sample	Analyte	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
cow fat	diflubenzuron	0.05 0.1	2 1	90 84	88-92	-	<0.05 (1)	DiPrima, 1976b, non-GLP (validation, DI-6836)
	CPU	0.05 0.1	2 1	93 83	92-94	-	<0.05 (1)	
	PCA	0.05 0.1	2 1	81 85	79-82	-	<0.05 (1)	
cow liver	diflubenzuron	0.05 0.1	2 1	88 83	79-96	-	<0.05 (1)	
	CPU	0.05 0.1	2 1	91 78	86-95	-	<0.05 (1)	
	PCA	0.05 0.1	2 1	71 87	63-79	-	<0.05 (1)	
cow kidney	diflubenzuron	0.05 0.1	2 1	83 96	80-85	-	<0.05 (1)	
	CPU	0.05 0.1	2 1	95 94	93-97	-	<0.05 (1)	
	PCA	0.05 0.1	2 1	78 87	71-85	-	<0.05 (1)	
cow muscle	diflubenzuron	0.05 0.1	2 1	97 81	93-100	-	<0.05 (1)	
	CPU	0.05 0.1	2 1	85 86	83-87	-	<0.05 (1)	
	PCA	0.05 0.1	1 1	102 88	-	-	<0.05 (1)	
chicken fat	diflubenzuron	0.05 0.1	2 1	85 87	77-93	-	<0.05 (1)	
	CPU	0.05 0.1	2 1	91 79	87-94	-	<0.05 (1)	
	PCA	0.05 0.1	2 1	82 78	76-89	-	<0.05 (1)	
chicken kidney	diflubenzuron	0.05 0.1	2 1	81 72	79-82	-	<0.05 (1)	

Sample	Analyte	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
	CPU	0.05 0.1	2 1	84 102	80-87	-	<0.05 (1)	
	PCA	0.05 0.1	2 1	75 71	71-80	-	<0.05 (1)	
chicken liver	diflubenzuron	0.05 0.1	2 1	97 90	91-102	-	<0.05 (1)	
	CPU	0.05 0.1	2 1	74 76	70-78	-	<0.05 (1)	
	PCA	0.05	2	83	79-86	-	<0.05 (1)	
chicken muscle	diflubenzuron	0.05 0.1	2 1	84 87	78-89	-	<0.05 (1)	
	CPU	0.05 0.1	2 1	77 73	73-82	-	<0.05 (1)	
	PCA	0.05 0.1	2 1	78 71	74-81	-	<0.05 (1)	
cow milk	diflubenzuron	0.05 0.1	2 1	104 88	101-107	-	<0.05 (1)	
	CPU	0.05 0.1	2 1	90 88	89-90	-	<0.05 (1)	
	PCA	0.05 0.1	2 1	87 83	-	-	<0.05 (1)	
chicken eggs	diflubenzuron	0.05 0.1	2 1	92 78	89-94	-	<0.05 (1)	
	CPU	0.05 0.1	2 1	89 86	88-89	-	<0.05 (1)	
	PCA	0.05 0.1	1 1	72 76	-	-	<0.05 (1)	
fish	diflubenzuron	0.05 0.1	4 3	101 106	94-107 100-115	5.3 7.6	<0.05 (1)	
	CPU	0.05 0.1	4 3	94 97	85-100 87-109	8.2 12	<0.05 (1)	
	PCA	0.05 0.1	2 1	91 88	80-102 -	-	<0.05 (1)	
sheep muscle	diflubenzuron	0.05 0.5	3 1	97 86	80-112	17	<0.05 (3)	Escobar, 1978, non-GLP (feeding study, DI-6840)
sheep liver	diflubenzuron	0.05 0.1 1	3 1 2	97 77 74	78-120 - 72-76	22	<0.05 (4)	
sheep kidney	diflubenzuron	0.05 0.5	2 1	87 90	82-92	-	<0.05 (1)	
sheep milk	diflubenzuron	0.05 0.5	1 1	84 96	-	-	<0.05 (1)	
modification A								Escobar, 1978 non-GLP (feeding study, DI-6840) *ad hoc* modification
sheep fat	diflubenzuron	0.05 0.5	4 1	96 75	78-123	20	<0.05 (4)	

De Wilde (1978a, non-GLP) described the determination of CPU in mushrooms, compost and casing (Method G). Homogenized samples were blended with ethyl acetate and sodium sulfate. The filtered extract was evaporated to dryness and the residue dissolved in acetone for mushrooms or ACN for compost and casing. Extracts of compost and casing were washed with hexane, the ACN phase was evaporated to dryness and the residue dissolved in acetone. The acetone solutions were purified on a silica gel column, evaporated to dryness and the residue redissolved in ACN. The CPU in the extract was derivatized with HFBA at 45°C and the product (CPHFBA) determined by GC-ECD (Carbowax 20-M column).

The method was slightly modified by De Wilde (1978b, non-GLP) by using a longer clean-up column and different GC conditions.

Validation data for mushrooms and compost are shown in Table 82. The derivatization efficiency and selectivity in the presence of PCA were not verified.

Table 82. Recoveries of CPU from mushrooms and compost by GC-ECD method G. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike mg/kg	n	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
original method								
mushrooms	0.01	0.01	1	82	-	-	<0.003-0.048 (9)	De Wilde, 1978a, non-GLP (residue trial, DI-3410)
		0.04	1	93	-	-		
		0.06	1	83	-	-		
		0.1	1	92	-	-		
		0.4	1	94	-	-		
		0.6	1	89	-	-		
		1.1	1	96	-	-		
compost	0.06	0.06	2	88	82-94	-	<0.018-0.024 (11)	
		0.1	1	75	-	-		
		0.4	1	78	-	-		
		0.6	2	80	77-83	-		
		1	1	84	-	-		
		2	1	73	-	-		
		4	1	82	-	-		
		10	1	77	-	-		
modification A								
mushrooms	0.02	0.02	3	105	90-124	17	<0.006-0.008 (12)	De Wilde, 1978b, non-GLP (residue trial, DI-1309) *ad hoc* modification
		0.04	3	70	48-90	30		
		0.06	3	79	23-121	64		
		0.2	1	99	-	-		
		0.4	1	94	-	-		
		0.6	1	110	-	-		
		1.0	1	102	-	-		
		1.2	1	122	-	-		
mushrooms	0.01	0.01	2	80	80-80	-	<0.003-0.006 (13)	De Wilde, 1979b, non-GLP (residue trial, DI-3409) *ad hoc*, De Wilde, 1978a
		0.04	2	83	78-88	-		
		0.06	3	84	73-88	12		
		0.1	1	80	-	-		
		0.4	1	82	-	-		
		0.6	1	91	-	-		
		1.0	1	89	-	-		
		2.0	1	90	-	-		
compost	0.02	0.02	1	60	-	-	<0.006-0.017 (8)	De Wilde, 1978b, non-GLP (residue trial, DI-1309) *ad hoc* modification
		0.03	1	81	-	-		
		0.05	1	81	-	-		
		0.1	1	86	-	-		
		0.3	1	95	-	-		
		0.5	1	89	-	-		
		1	1	98	-	-		
		2	1	99	-	-		
		4	1	78	-	-		
		6	1	83	-	-		

Method LAI 3-86-9, 01 March 1988 (Duphar, 1988g, non-GLP) was applied to the determination of CPU in apples, sugar beets, sugar beet leaves, grass, spinach, onions, wheat, mushrooms, protective soil layers, compost, cow and calf dung. Homogenized samples (50-100 g) were blended with ethyl acetate and sodium sulfate. The filtered extract was evaporated to dryness and the residue dissolved in acetone/petroleum ether (5+25) for apples, onions, wheat and mushrooms or in ACN for all other samples. The ACN solution was washed with hexane, and the solvent changed to acetone/petroleum ether, 5+25, for clean-up on a silica gel column. The eluate was evaporated to dryness and the residue dissolved in hexane, then transferred to ACN. After derivatization with HFBA at room temperature, the CPHFBA was determined by GC-ECD as above.

Uhden *et al.* (1995, non-GLP) modified the method for mushrooms by changing the amount of homogenized sample to 25 g, changing the filtration apparatus and the volume of derivatization reagent and using a DB-1 column. They renamed the method CARDC-1248-CPU.

Allan and Thus (1996, non-GLP) modified the method for mushrooms again. The filtered extract was evaporated to dryness and taken up in ACN. This solution was cleaned up by washing with hexane. The ACN phase was evaporated to dryness, and the residue dissolved in acetone and purified on a silica gel column. Determination was on a DB-17 column.

Rose (1999a, GLP) modified the method for sugar-containing commodities by introducing a water and a hexane/ACN partition step. The filtered extract was partitioned against water. The ethyl acetate phase was filtered through sodium sulfate and evaporated to dryness, and the residue dissolved in hexane and partitioned into ACN before transfer to acetone/petroleum ether and clean-up as before. Determination was on a DB-1701 column.

Several further *ad hoc* modifications were made by changing the amount of homogenized sample, the volume of the extraction solvent, the filtration apparatus, volumes in column chromatography and of derivatization reagent, and by using DB-17, DB-1701 or DB-5 columns.

Validation data are shown in Table 83.

Table 83. Recoveries of CPU from crop samples by modifications of method LAI 3-86-9. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
modification A, CARDC-1248-CPU								Uhden *et al.*, 1995
mushrooms	0.01	0.01	3	106	100-112	5.9	<0.003 (3)	Uhden *et al.*, 1995, non-GLP (validation, RP-96015)
		0.05	3	92	85-99	7.7		
		0.5	3	89	84-97	7.8		
mushrooms	0.01	0.1	16	90	70-106	10	<0.01-0.01 (16)	Gaydosh, 1999c, GLP (residue trial, RP-96015)
		1	16	82	70-101	12		
modification B								Allan and Thus, 1996
mushrooms	0.01	0.1	2	88	85-90	-	<0.003 (4)	Allan and Thus, 1996, non-GLP (residue trial, DI-9441)
modification C								Rose, 1998a
mushrooms	0.01	0.01	2	97	88-106	-	<0.003 (2)	Rose, 1998a, GLP (validation, RP-97004)
		0.1	2	95	86-104	-		
mushrooms	0.01	0.02	32	97	68-125	16	<0.01 (16)	Gaydosh, 1998a, GLP (residue trial, RP-97004)
modification D								Rose, 2000b
mushrooms (freshly spiked aged samples)	0.01	0.1	14	90	71-125	18	<0.003-0.010 (7)	Rose, 2000b, GLP (storage stability, RP-98024)
modification E								Rose, 1998b
lettuce	0.01	0.01	2	97	95-98	-	<0.003 (2)	Rose, 1998b, GLP (validation, RP-95018)
		0.05	2	111	110-112	-		
		0.1	2	107	107-107	-		
lettuce	0.01	0.03	26	85	71-115	14	<0.003-0.009 (13)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
turnip tops	0.01	0.01	2	109	108-110	-	0.003 (2)	Rose, 1998b, GLP (validation, RP-95018)
		0.05	2	100	99-100	-		
		0.1	2	89	89-89	-		
turnip tops	0.01	0.03	24	98	69-118	14	<0.003-0.033 (12)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
turnip roots	0.01	0.01	2	105	104-105	-	0.0057 (2)	Rose, 1998b, GLP (validation, RP-95018)
		0.05	2	109	108-110	-		
		0.1	2	104	103-105	-		
turnip roots	0.01	0.03	24	102	56-117	16	<0.003-0.056 (12)	Gaydosh, 1999d, GLP

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD_r	Controls mg/kg	Reference
								(rotational crops, RP-95018)
wheat forage	0.01	0.01 0.05 0.1	2 2 2	105 84 85	104-106 81-86 82-87	- - -	<0.003 (2)	Rose, 1998b, GLP (validation, RP-95018)
wheat forage	0.01	0.03	18	95	75-117	13	<0.003-0.009 (9)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
wheat grain	0.01	0.01 0.05 0.1	2 2 2	83 81 94	82-84 80-81 93-94	- - -	<0.003 (2)	Rose, 1998b, GLP (validation, RP-95018)
wheat grain	0.01	0.03	20	87	68-115	16	<0.003-0.006 (10)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
wheat hay	0.05	0.01 0.05 0.1	2 2 2	128 83 95	127-128 83-83 93-96	- - -	<0.015 (2)	Rose, 1998b, GLP (validation, RP-95018)
wheat hay	0.05	0.03	22	90	61-117	18	<0.003-0.003 (11)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
wheat straw	0.01	0.01 0.05 0.1	2 2 2	74 77 69	72-75 76-77 69-69	- - -	<0.003 (2)	Rose, 1998b, GLP (validation, RP-95018)
wheat straw	0.01	0.03	22	99	78-120	13	<0.003-0.008 (11)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
modification F								Hathcock and Ruzo, 1998a
lettuce (freshly spiked aged samples)	0.01	0.1	12	100	71-127	18	<0.003 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
turnip roots (freshly spiked aged samples)	0.01	0.1	12	93	81-104	7.8	<0.003-0.009 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
wheat grain (freshly spiked aged samples)	0.01	0.1	12	82	67-103	13	<0.003 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
wheat hay (freshly spiked aged samples)	0.01	0.1	12	92	76-117	17	<0.003-0.038 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
modification G								Nishioka *et al.*, 1997
pear	0.001	0.001 0.005 0.01	2 2 2	94 89 91	84-105 79-99 88-93	- - -	<0.0003 (1)	Nishioka *et al.*, 1997, GLP (validation, RP-96025)
pear	0.001	0.005	4	80	65-104	21	<0.001 (2)	Gaydosh and Rose, 1999 GLP (residue trial, RP-96025)
pear	0.001	0.01 0.02	11 2	82 94	65-115 89-99	21 -	<0.001-0.002 (14)	Dorschner and Gaydosh, 2000, GLP (residue trial, IR-4)
pear (freshly spiked aged samples)	0.001	0.10	8	97	87-108	7.4	<0.001 (4)	Nishioka *et al.*, 1997, GLP (storage stability, RP-96025)
modification H								Nishioka and Rose, 1997b
whole rice and rice grain	0.001	0.001 0.005 0.01	4 4 4	107 87 91	104-111 84-90 85-95	3.1 3.2 7.4	<0.0003 (4)	Nishioka and Rose, 1997b, GLP (validation, RP-95028)
whole rice	0.001	0.03	2	82	76-88	-	<0.001 (2)	Gaydosh *et al.*, 1997b, GLP (processing, RP-96013)
whole rice	0.001	0.03	2	89	71-107	-	<0.001 (2)	Gaydosh, 1998b, GLP (processing, RP-95028)
rice, grain	0.001	0.03	10	98	76-118	18	<0.001 (7)	Gaydosh *et al.*, 1997a, GLP (residue trial, RP-96012)
rice, grain	0.001	0.001	6	101	65-142	26	<0.001 (5)	Gaydosh and Puhl, 1998 (residue trial, RP-98029)
rice grain (freshly spiked aged samples)	0.001	0.1	12	77	69-85	8.0	<0.0003-0.033 (6)	Hathcock and Ruzo, 1998b, GLP (storage stability, RP-97026)
polished rice	0.001	0.001 0.005	2 2	95 93	94-95 92-94	- -	0.000 (2)	Nishioka and Rose, 1997b, GLP (validation, RP-95028)

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
		0.01	2	118	117-118	-		
white rice	0.001	0.03	2	78	71-85	-	<0.001 (2)	Gaydosh *et al.*, 1997b, GLP (processing, RP-96013)
white rice	0.001	0.03	2	82	82-83	-	<0.001 (2)	Gaydosh, 1998b, GLP (processing, RP-95028)
rice, straw	0.01	0.01	2	74	74-74	-	<0.003 (3)	Nishioka and Rose, 1997b, GLP (validation, RP-95028)
rice straw	0.01	0.03	10	86	72-119	19	<0.01 (7)	Gaydosh *et al.*, 1997a, GLP (residue trial, RP-96012)
rice, straw	0.01	0.01 0.02	6 2	88 74	40-149 72-77	48 -	<0.01 (5)	Gaydosh and Puhl, 1998 (residue trial, RP-98029)
rice, straw	0.01	0.03	2	92	96-88	-	<0.01 (2)	Gaydosh *et al.*, 1997b, GLP (processing, RP-96013)
rice, straw	0.01	0.03	3	92	87-100	7.9	<0.01 (2)	Gaydosh, 1998b, GLP (processing, RP-95028)
rice straw (freshly spiked aged samples)	0.01	0.1	12	105	69-121	17	<0.003-0.040 (6)	Hathcock and Ruzo, 1998b, GLP (storage stability, RP-97026)
rice, hulls	0.005	0.005 0.01	4 4	96 78	91-101 76-80	5.0 2.6	<0.0015 (2)	Nishioka and Rose, 1997b, GLP (validation, RP-95028)
rice, hulls	0.005	0.03	2	80	72-89	-	<0.005 (2)	Gaydosh *et al.*, 1997b, GLP (processing, RP-96013)
rice, hulls	0.005	0.03	2	104	102-105	-	<0.005 (2)	Gaydosh, 1998b, GLP (processing, RP-95028)
rice hulls (freshly spiked aged samples)	0.005	0.1	12	75	51-99	16	<0.0015-0.010 (6)	Hathcock and Ruzo, 1998b, GLP (storage stability, RP-97026)
rice, bran	0.005	0.005 0.01	4 4	95 98	92-100 83-113	3.7 17	<0.0015 (2)	Nishioka and Rose, 1997b, GLP (validation, RP-95028)
rice, bran	0.005	0.03	2	118	116-121	-	<0.005 (2)	Gaydosh *et al.*, 1997b, GLP (processing, RP-96013)
rice, bran	0.005	0.03	2	80	78-82	-	<0.005 (2)	Gaydosh, 1998b, GLP (processing, RP-95028)
rice bran (freshly spiked aged samples)	0.005	0.1	12	96	85-112	8.9	<0.0015-0.018 (6)	Hathcock and Ruzo, 1998b, GLP (storage stability, RP-97026
modification I								Rose, 1999b
pepper	0.01	0.01 0.1 0.6	6 5 6	87 92 95	76-100 81-100 80-110	11 9.7 16	<0.003 (4)	Rose, 1999b, GLP (validation, RP-97016)
pepper	0.01	0.2	10	102	70-132	23	<0.01 (9)	Gaydosh and Puhl, 1999, GLP (residue trial, RP-97016)
pepper (freshly spiked aged samples)	0.01	0.2	9	88	69-109	15	<0.003-0.007 (5)	Rose, 1999b, GLP (storage stability, RP-97016)
modification J								Rose, 1999a
peach	0.005	0.005 0.01	2 2	107 94	107-107 94-94	- -	<0.0015 (2)	Rose, 1999a, GLP (validation, RP-97008)
peach	0.005	0.05 0.1	10 1	92 87	62-117 -	19 -	<0.005-0.034 (6)	Gaydosh, 1999b, GLP (residue trial, RP-97008)

Modification H for the determination of CPU in rice (Nishioka and Rose, 1997b, GLP) was proposed as an enforcement method. Vithala and Dematteo (1998, GLP) reported on its selectivity in the presence of other pesticides. Since CPU is determined by GC-ECD, only halogenated pesticides were selected (methoxychlor, 2,4-D, naled, picloram, MCPA, metolachlor, permethrin, triclopyr, chlorpyrifos-methyl, fenoxaprop-ethyl, propiconazole, lambda-cyhalothrin, paraquat, flutolanil, fipronil, benoxacor, quichlorac, chlorpropham (CIPC) and methyl bromide). The derivatization is followed by separation between an aqueous and a hexane layer. Of the halogenated pesticides tested, only four were found in the hexane layer together with CPU. By GC-FID, the retention time of these

HFBA-derivatized and underivatized pesticides was different from the retention time of derivatized CPU. The authors concluded that selectivity in the presence of halogenated pesticides that are registered for use on rice in the USA was satisfactory.

Batorewicz (1998, GLP) reported an independent validation of modification H in which an Osterizer blender was used instead of a Warring blender. Homogenized rice grain fortified at 0.02 mg/kg CPU gave recoveries between 0 and 28%. The authors concluded that the purification on hand-packed silica gel columns was not reproducible.

Cassidy (1998a, non-GLP) reported another independent validation of modification H in which a different GC temperature programme was used. Homogenized rice grain fortified at 0.001 and 0.02 mg/kg CPU gave mean recoveries of 102% and 98% respectively, and rice straw fortified at 0.01 and 0.8 mg/kg CPU gave 86% and 100% respectively (all duplicate analyses). Untreated duplicate control samples contained <0.001 mg/kg CPU in rice grain and <0.001-0.011 mg/kg in rice straw.

Jalali and Hiler (1997, GLP) compared modification H with HPLC and LSC in [^{14}C]diflubenzuron-treated rice plants from the metabolism study described by Walsh (1997). Details of analyses by the reference method HPLC-LSC are given above in the description of the similar comparison with modification E of method LAI 3-86-6.

The results are shown in Table 84. The CPU residue method showed no bias (<5.7%) for incurred radioactive residues in the presence of 0.0003 mg/kg diflubenzuron in rice grain or 0.37 mg/kg diflubenzuron in rice straw. The chosen samples did not contain PCA. The CPU levels in the present study and the metabolism study were comparable: 0.015 and 0.23-0.25 mg/kg in the present study and 0.015 and 0.27 mg/kg in the original study, indicating no storage instability for a period of 12 months.

Uhden *et al.* (1995, non-GLP) reported on the selectivity for CPU of modification A (CARDC-1248-CPU) in the presence of PCA. In spiked mushrooms, the average PCA recovery was 5.3% ± 5.1% (level not reported), while the concurrent average CPU recovery was 92% ± 7.1% (at 0.05 mg/kg CPU). The analytical results were however not reported.

Batorewicz (2000a, non-GLP) reported a revision of modification H. Hand-packed columns were replaced by commercially packed silica SPE cartridges, resulting in a fast and reproducible clean-up. They also investigated the use of synthesized CPHFBA standards instead of *in situ*-derivatized standards. The efficiency of conversion of CPU to CPHFBA under various conditions was less than quantitative (71%-91%), whereas the efficiency for PCA to CPHFBA was 98%-105%. The efficiency of hydrolysis of CPU to PCA was poor (11%-37% for 2-4 hours in 1-12 M HCl at 90°C). According to the author it is not possible to use synthesized CPHFBA standards, because the efficiency of derivatization of CPU is lower than that of PCA, and CPU cannot easily be hydrolysed to PCA.

A confirmatory technique was not proposed for rice and the method is not generally available (unpublished).

Table 84. Comparison of CPU methods in ^{14}C-treated rice plants.

Sample	Treatment (kg ai/ha)	TRR mg/kg diflubenzuron equivalents (Walsh, 1997)	TRR mg/kg diflubenzuron equivalents (Jalali and Hiler, 1997)	Distribution in extracts (Walsh, 1997)	CPU (mg/kg diflubenzuron equivalents) HPLC-LSC (Jalali and Hiler, 1997)	CPU (mg/kg diflubenzuron equivalents) GC-ECD (Jalali and Hiler, 1997)
grain	0.28	0.091	0.091	diflubenzuron 0.2% CPU 17% PCA not detected	0.015	0.015

Sample	Treatment (kg ai/ha)	TRR mg/kg diflubenzuron equivalents (Walsh, 1997)	TRR mg/kg diflubenzuron equivalents (Jalali and Hiler, 1997)	Distribution in extracts (Walsh, 1997)	CPU (mg/kg diflubenzuron equivalents) HPLC-LSC (Jalali and Hiler, 1997)	CPU (mg/kg diflubenzuron equivalents) GC-ECD (Jalali and Hiler, 1997)
straw	0.28	1.0	0.88	diflubenzuron 36%; CPU 26%; PCA not detected	0.24	0.23

Method RES025, 14 December 1988 (Duphar, 1988j, non-GLP) was developed for the determination of CPU in soil. After addition of water, 50 g soil was blended with ethyl acetate and sodium sulfate. After filtration, part of the extract was evaporated to dryness and the residue redissolved in ACN, washed with hexane, transferred to acetone and cleaned up on a silica gel column. CPU in the eluate was derivatized with HFBA and determined by GC on a 3% Carbowax 20-M column.

Kramer (1990, 1991, 1992, GLP) modified the method *ad hoc* by changing elution volumes in the clean-up step and the injection temperature of the GC.

Ver Hey (1991a,b, GLP) changed the amount of HFBA and used a different column (DB-1).

Although validation reports exist (reports 56635/34/90 and 56635/35/90) they were not available to the reviewer. Validation data are shown in Table 85. The derivatization efficiency for CPU and the selectivity in the presence of diflubenzuron and PCA were not verified.

Table 85. Recoveries of CPU from soil by modifications of method RES025. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
modification A								Kramer, 1990
loam soil	0.01	0.01 0.1 1	2 2 2	99 104 94	99-99 101-106 93-95	- - -	<0.01 (2)	Kramer, 1990, GLP (validation)
loam soil	0.01	0.1 0.2 0.4	2 12 7	82 85 92	80-84 73-97 85-100	- 10 5.4	<0.01 (21)	Kramer, 1990, GLP (field dissipation study)
loam soil (freshly spiked aged samples)	0.01	0.5	8	87	72-101	9.6	<0.01 (8)	Kramer, 1990, GLP (storage stability)
sandy soil	0.1	0.01 0.1 1	2 2 2	77 88 105	76-78 86-89 103-107	- - -	<0.01 (2)	Kramer, 1991, GLP (validation)
sandy soil	0.01	0.1 0.2 0.4	6 8 4	85 89 83	73-100 84-94 74-91	12 4.0 9.1	<0.01 (50)	Kramer, 1991, GLP (field dissipation study)
sandy soil (freshly spiked aged samples)	0.01	0.4 0.5 5 6	3 4 1 1	87 91 114 106	79-98 82-95 - -	11 6.5 - -	<0.01 (8)	Kramer, 1991, GLP (storage stability)
silt loam	0.01	0.01 0.03 0.05 0.1 0.3	8 2 2 2 2	104 92 101 103 80	84-114 87-97 100-101 101-105 80-80	10 - - - -	<0.01 (4)	Kramer, 1992, GLP (validation)
silt loam	0.01	0.01 0.1	12 12	91 87	70-105 72-102	11 9.3	<0.01 (32)	Kramer, 1992, GLP (field dissipation study)
silt loam (freshly spiked aged samples)	0.01	0.5	5	80	66-93	12%	<0.01 (5)	Kramer, 1992, GLP (storage stability)
modification B								Ver Hey, 1991a

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
sandy loam soil	0.01	0.01 0.02 0.05 0.1 0.5 1 2	21 24 13 7 5 1 1	70 72 74 52 74 56 71	43-103 42-110 50-92 41-64 62-83 - -	27 29 17 15 13 - -	<0.01 (72)	Ver Hey, 1991a, GLP (field dissipation study)
sandy loam soil (freshly spiked aged samples)	0.01	0.1	5	73	59-92	23	<0.01 (23)	Ver Hey, 1991a, GLP (storage stability)
silty loam soil	0.01	0.01 0.02 0.05 0.1 0.5 1 2	18 18 11 12 6 1 2	61 61 68 52 54 58 54	41-93 31-84 57-81 40-73 44-70 - 42-67	27 21 13 20 22 - -	<0.01-0.01 (67)	Ver Hey, 1991b, GLP (field dissipation study)
silty loam soil (freshly spiked aged samples)	0.01	0.1	4	81	53-116	33	<0.01 (16)	Ver Hey, 1991b, GLP (storage stability)

In GC-ECD method RES027, 11 January 1989 (Duphar, 1989a) for the determination of PCA in oranges, homogenized oranges were ground with dichloromethane/12 M NaOH (200+5) and anhydrous sodium sulfate for 10 min. After filtration and addition of 0.5 M HCl, the dichloromethane layer was washed with HC1 and discarded. The aqueous phase was washed with hexane and adjusted to pH >12 with 12 M NaOH, and PCA was partitioned into hexane. The PCA was derivatized with HFBA and determined on a DB-17 column.

Van Zijtveld *et al.* (1989, non-GLP) modified the method for citrus fruits. The sample was incubated for 1 hour with water/12.5 M NaOH (5+10) before the addition of dichloromethane and sodium sulfate and a fivefold amount of sample was derivatized with HFBA with the same final volume as in the original method.

A validation report was not available. The limited validation data are shown in Table 86. The derivatization efficiency for PCA and selectivity in the presence of diflubenzuron and CPU were not verified. Recoveries were low and variable, and the method is not considered capable of determining PCA in citrus fruits.

Table 86. Recoveries of PCA from crop samples by method RES027 or a modification. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference (type of trial) method version, GLP
modification A								Van Zijtveld *et al.*, 1989
oranges	0.001	0.001 0.005 0.01	11 1 1	28 41 38	20-37 - -	22 - -	<0.0003-0.00032 (24)	Van Zijtveld *et al.*, 1989; non-GLP, (residue trial; storage stability, DI-7492)
grapefruit	0.001	0.001 0.005 0.01	4 2 1	26 40 46	13-39 35-45 -	54 - -	<0.0003 (6)	

Kane *et al.* (1996, non-GLP) described method CARDC-1248-PCA for the determination of PCA in mushrooms. The homogenized sample was refluxed for 30 min with 0.1 M HCl. After filtration and basification with NaOH (pH >10), the PCA was steam-distilled into 0.1 M HCl. The distillate was basified (pH >10) and PCA partitioned into hexane, derivatized with HFBA and determined by GC on a DB-1701 column. The method was also used for rice.

Validation data are shown in Table 87. The derivatization efficiency for PCA and selectivity in the presence of diflubenzuron and CPU were not verified. Recoveries from rice were low and the method is not considered capable of determining PCA in rice.

Table 87. Recoveries of PCA from mushrooms and rice by method CARDC-1248-PCA or a modification. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
mushrooms	0.01	0.01 0.05 0.1	3 3 3	82 81 81	77-86 78-85 78-83	5.9 4.3 3.5	<0.003-0.003 (2)	Kane *et al.*, 1996, non-GLP (validation, RP-96015)
mushrooms	0.01	0.1 1	16 16	80 77	69-87 71-88	5.5 6.4	<0.01-0.02 (16)	Gaydosh, 1999c, GLP (residue trial, RP-96015)
rice, grain	0.01	0.01 0.025	4 4	38 52	36-40 50-55	4.3 4.0	<0.01 (2)	Morgenstern, 1996a, GLP (validation, RP-95016)
rice, straw	0.01	0.01 0.025 0.05	5 4 4	27 21 27	12-45 7.6-32 6.9-46	57 61 81	0.007-0.015 (3)	Morgenstern, 1996a, GLP (validation, RP-95016)

Dijksman *et al.* (1990, GLP) reported method K for PCA in milk. 4-Bromoaniline was added to the milk as internal standard (250 µg/l), the milk was extracted with pentane, and the pentane extract with 0.07 M perchloric acid in water. The perchloric acid extract was extracted with diethyl ether/hexane (1:9) in the presence of potassium carbonate. The diethyl ether/hexane extract was extracted with ACN in the presence of 1% heptafluorobutyric acid (not the anhydride), to ensure complete partitioning of PCA into the ACN layer, which was derivatized with HFBA for 15 min at 60°C. The derivatized PCA was extracted with hexane. After clean-up by HPLC (silica gel column) the sample was analysed by GC on a DB-1 column. Quantification was based on the ratio between derivatized PCA and derivatized 4-bromoaniline using matrix-matched standards.

Selectivity in the presence of diflubenzuron, PCAA and CPU was good, as these components were separated from PCA in the extraction steps before derivatization.

Timmerman *et al.* (1992b, non-GLP) modified the method for PCA in goat liver. Liver samples were homogenized in skimmed milk (1:1; w/w) and extracted with ammonia. Further extraction and analysis was as above.

The authors analysed the extraction layers from the various steps in the protocol using liver spiked with [^{14}C]diflubenzuron, [^{14}C]CPU and [^{14}C]PCA. Up to the perchloric acid step 65% of the PCA was extracted while <0.5% of the diflubenzuron and CPU were extracted, indicating good selectivity for PCA. It was assumed by the authors that the extraction of PCAA was similar to that of CPU in view of its physical and chemical properties, but results were not reported.

Recoveries are shown in Table 88. These are not recoveries in the usual sense because an internal standard is used. Recoveries represent calibration errors, which is why they are scattered rather randomly above and below 100%. The derivatization efficiency for PCA and the recovery of the internal standard 4-bromoaniline were not verified.

Table 88. Recoveries of PCA from goat milk and liver by GC-ECD method K. The ranges of recoveries were not reported.

Sample	LOQ, mg/kg	Spike mg/kg	No. [1]	% Rec mean.	% RSD$_r$	Controls mg/kg	Reference
Milk, 0-10 µg/l calibration	0.001	0.0010 0.0025 0.0050	5 7 7	120 104 96	12 19 8.4	<0.0003 (1)	Dijksman *et al.*, 1990, GLP (validation)

Sample	LOQ, mg/kg	Spike mg/kg	No. [1]	% Rec mean.	% RSD_r	Controls mg/kg	Reference
Milk, 0-50 µg/l calibration	0.001	0.0010	5	110	14	<0.0003 (1)	
		0.0025	7	104	22		
		0.0050	7	98	8.4		
Modification A							Timmerman *et al.*, 1992b
Liver	0.01	0.01[2]	2[4]	104	-	na	Timmerman *et al.*, 1992b, non-GLP, (validation, DI-7721)
		0.04[3]	3	114	4.0		

[1] The numbers represent the number of calibration curves with which single samples were analysed

[2] Liver homogenate contained 0.05 mg/kg each of diflubenzuron, CPU and PCAA

[3] Liver homogenate contained 0.2 mg/kg each of diflubenzuron, CPU and PCAA

[4] one outlier discarded

na: not analysed

In method CARDC-1283-PCA, 24 July 1996 (Morgenstern, 1996b, non-GLP) for the determination of PCA in animal tissues, homogenized tissues are refluxed with 0.1 M HCl for 30 min. The filtered extract is basified (pH >10) and steam-distilled into HCl. The distillate is basified (pH >10) and the PCA partitioned into hexane and derivatized with HFBA. The timing of the derivatization is critical and any water present after derivatization causes lower recoveries. The PCA derivative is determined by GC-ECD on a DB-1701 column. Quantification is based on *in situ* derivatized PCA standards (not matrix-matched).

Validation data are shown in Table 89. The efficiency of hydrolysis and derivatization of PCA and the selectivity in the presence of diflubenzuron, CPU and PCAA were not verified.

Table 89. Recoveries of PCA from cattle tissues and milk by GC-ECD method CARDC-1283-PCA. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike mg/kg	No.	% Rec mean.	% Rec range	% RSD_r	Controls mg/kg	Reference
Beef kidney	0.001	0.001	4	65	50-78	24	<0.0003 (2)	Morgenstern and Early, 1996, GLP (validation)
		0.005	4	57	56-58	2.1		
		0.01	4	60	56-64	5.3		
Beef liver	0.01	0.01	6	82	72-100	14	<0.003-0.0033 (3)	
Dairy milk	0.005	0.005	6	85	81-90	3.6	<0.0015 (3)	
		0.01	6	85	78-90	5.9		
Beef muscle	0.001	0.001	6	66	49-76	17	<0.0003-0.0009 (3)	
		0.005	6	69	60-78	9.7		
		0.01	6	74	65-83	10		
Beef omental fat	0.001	0.001	4	86	62-112	26	<0.0003 (2)	
		0.005	4	74	67-80	8.7		
		0.01	4	73	68-78	6.0		
Beef renal fat	0.001	0.001	6	84	68-103	14	<0.0003 (3)	
		0.005	6	77	74-82	3.9		
		0.01	6	78	67-82	6.8		

Uhden *et al.* (1996, non-GLP) described method CARDC-1248-DFBA for the determination of DFBA in mushrooms. Homogenized sample was blended with 4N sulfuric acid/ACN/water (15+90+50). The filtered extract was purified on an "Extrelut" column and the eluate transferred to ethyl acetate and evaporated to dryness. The DFBA was derivatized with 0.2% pentafluorobenzyl bromide (PFBBr) in acetone (30 min 50°C). The solvent was evaporated and the residue in toluene/hexane (1+6) purified on a silica gel column for analysis.

Validation data are shown in Table 90. The derivatization efficiency for DFBA and selectivity in the presence of diflubenzuron were not verified.

Table 90. Recoveries of DFBA from mushrooms by method CARDC-1248-DFBA. Numbers in parentheses are numbers of control samples.

LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
0.01	0.01	3	96	77-110	18	<0.003 (2)	Uhden *et al.*, 1996, (validation,)
	0.05	3	89	88-90	1.1		
	0.1	3	103	99-108	4.3		
	0.50	3	105	101-108	3.4		
0.01	0.1	16	98	76-122	15	<0.01-0.03 (5) variety Amycel 2600: 0.16-0.95 (7)	Gaydosh, 1999c, GLP (residue trial, RP-96015)
	1	16	93	82-112	9.6		

Method RES031, 21 March 1989 (Duphar, 1989b) was applied to the GC-ECD determination of DFBA in soil. DFBA was extracted from 50 g soil with 10% sulfuric acid in water in an ultrasonic bath for 5 min and blended with diethyl ether, sodium sulfate and filter pulp. After filtration, part of the diethyl ether layer was evaporated to dryness after the addition of 50 µl tetradecane. The residue was redissolved in 1% PFBBr, derivatized for 30 min at 50°C, transferred to toluene:hexane (9+1) and cleaned up on a silica gel column. Alternatively the residue may be redissolved in 0.2% PFBBr, in which case the clean-up step may be omitted. DFBA was determined on a DB-1 column. The version of 26 April 1989 (Duphar, 1989c) does not differ except in some textual changes.

Kramer (1990, 1991, GLP) changed the injection volume and detector temperature for GC and the standard concentrations for the calibration curve.

Ver Hey (1991a,b, GLP) used dodecane instead of tetradecane and the alternative derivatization method.

Although validation reports exist (reports 56635/34/90 and 56635/35/90) they were not available to the reviewer. Validation data are shown in Table 91. The derivatization efficiency of DFBA and the selectivity in the presence of diflubenzuron, CPU and PCA were not verified.

Table 91. Recoveries of DFBA from soil by modifications of method RES031. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
modification A								Kramer, 1990, 1991
loam soil	0.01	0.01	2	114	109-120	-	<0.01 (2)	Kramer, 1990, GLP (validation)
		0.1	2	97	92-102	-		
		1.0	2	98	83-113	-		
loam soil	0.01	0.01	1	113	-	-	<0.01 (15)	Kramer, 1990, GLP (field dissipation study)
		0.05	2	110	102-119	-		
		0.07	1	105	-	-		
		0.1	3	108	100-114	6.9		
		0.2	1	106	-	-		
		0.4	5	92	70-110	16		
		0.5	2	87	80-94	-		
loam soil (freshly spiked aged samples)	0.01	0.5	8	90	80-104	8.4	<0.01 (8)	Kramer, 1990, GLP (storage stability)
sandy soil	0.01	0.01	2	102	97-108	-	<0.01 (2)	Kramer, 1991, GLP (validation)
		0.1	2	99	91-108	-		
		1	2	97	95-99	-		
sandy soil	0.01	0.01	1	80	-	-	<0.01 (45)	Kramer, 1991, GLP (field dissipation study)
		0.02	1	85	-	-		
		0.05	3	109	106-113	3.2		
		0.1	6	102	82-116	14		
		0.2	3	98	73-113	22		
		0.4	1	101	-	-		

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
		0.5	3	100	78-114	19		
sandy soil (freshly spiked aged samples)	0.01	0.2 0.4 0.5 0.6 5	2 1 3 2 1	85 84 107 93 101	84-86 - 94-119 90-95 -	- - 12 - -	<0.01 (8)	Kramer, 1991, GLP (storage stability)
silt loam	0.01	0.01 0.03 0.05 0.1 0.3	8 2 2 2 2	103 111 104 87 79	74-119 110-111 102-106 87-87 78-81	16 - - - -	<0.01 (4)	Kramer, 1992, GLP (validation)
silt loam	0.01	0.01 0.1	11 10	116 97	97-128 83-112	7.9 11	<0.01 (28)	Kramer, 1992, GLP (field dissipation study)
silt loam (freshly spiked aged samples)	0.01	0.5	5	96	89-107	8.4	<0.01 (5)	Kramer, 1992, GLP (storage stability)
modification B								Ver Hey, 1991a,b
sandy loam soil	0.01	0.01 0.02 0.05 0.1 0.5 1 2	21 24 14 7 4 1 1	94 100 98 96 98 116 94	77-122 84-123 74-122 84-117 81-109 - -	12 10 13 14 13 - -	<0.01 (72)	Ver Hey, 1991a, GLP (field dissipation study)
sandy loam soil (freshly spiked aged samples)	0.01	0.1	5	83	70-96	12	<0.01 (23)	Ver Hey, 1991a, GLP (storage stability)
silty loam soil	0.01	0.01 0.02 0.05 0.1 0.5 1 2	20 19 10 12 6 1 2	93 98 101 101 102 87 100	71-130 69-122 86-125 92-118 82-118 - 90-110	15 15 12 9.1 14 - -	<0.01-0.02 (70)	Ver Hey, 1991b, GLP (field dissipation study)
silty loam soil (freshly spiked aged samples)	0.01	0.1	4	111	102-127	11	<0.01 (16)	Ver Hey, 1991b, GLP (storage stability)

GC-MS methods

De Wilde (1978a, non-GLP) described the determination of DFBA in mushrooms and compost/casing. Homogenized sample was blended with anhydrous sodium sulfate, 4N sulfuric acid/diethylether (1+5) and filter pulp. The filtered extract was concentrated and DFBA methylated with 0.5 M diazomethane in diethyl ether. Excess diazomethane was removed by addition of acetic acid. After dilution with diethyl ether, the solution was analysed by GC-MS (5% SE-30 column, m/z 141). De Wilde (1978b, non-GLP) slightly modified the method by using a different amount of filter pulp.

Later versions of this method were named LAI 3-86-10, 10 Feb 1987 (Duphar, 1987a). Modifications were the adjustment to pH <3 after filtration, further concentration, use of more diazomethane, longer methylation time, adjustment to pH <4 after addition of acetic acid, use of hexane for dilution and different GC conditions (fused silica, CP Wax 51, m/z 172, 141, 113). Allan and Thus (1996) used a DB-1 column. They confirmed the identity by measuring the ratio of 141:172 (7:1).

Validation data are shown in Table 92. The derivatization efficiency for DFBA and selectivity in the presence of diflubenzuron were not verified.

Table 92. Recoveries of DFBA from mushrooms and compost by GC-MS method LAI 3-86-10 and minor modifications. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
mushrooms	0.05	0.05	2	115	102-128	-	<0.015-0.053 (18)	De Wilde, 1978a, non-GLP
		0.1	2	112	98-126	-		(residue trial, DI-3410)
		0.5	2	92	90-95	-		De Wilde, 1978b, non-GLP
		1	2	88	85-91	-		(residue trial, DI-1309)
		5	2	90	74-105	-		
		10	2	76	58-93	-		
		25	2	93	87-99	-		
mushrooms	0.01	0.01	1	100	-	-	<0.003-0.005 (12)	De Wilde, 1979a, non-GLP
		0.04	1	93	-	-		(residue trial, DI-3409)
		0.06	1	102	-	-		
		0.1	1	102	-	-		
		0.6	1	101	-	-		
		1	1	102	-	-		
		2	1	95	-	-		
mushrooms	ns	0.1	1	97	-	-	0.000 (4)	Allan and Thus, 1996, non-GLP
		0.5	2	115	110-120	-	(value as reported)	(residue trial, DI-9441)
compost	0.05	0.05	2	121	118-124	-	<0.015-0.15 (16)	De Wilde, 1978a, non-GLP
		0.10	2	104	90-119	-		(residue trial, DI-3410)
		0.50	2	102	101-103	-		De Wilde, 1978b, non-GLP
		1.0	2	90	86-94	-		(residue trial, DI-1309)
		5.0	2	108	102-114	-		
		10	2	100	99-101	-		
		25	2	96	93-99	-		

Ruzo and Rose (1998, GLP) described GC-ID-MS method PTRL 645W (5 June 1997) for PCA in rice samples. [^{13}C]PCA was added as an internal standard to the homogenized sample. The sample was hydrolysed for 30 min at 60°C with 0.1 M HCl. After filtration and basification with NaOH (pH >12), the PCA was partitioned into hexane. After re-extraction with 0.1 M HCl, partitioning into hexane and clean-up on a Florisil column, PCA was derivatized with HFBA and determined by GC-ID-MS (SPB-1701 column; electron impact; single ion monitoring). Quantification was based on the combined peak areas at m/z 323 and 126 for [^{12}C]PCA relative to the combined areas at m/z 329 and 132 for [^{13}C]PCA.

Minor *ad hoc* modifications (Nishioka *et al.*, 1997; Rose, 1998a,b, 1999a-d,f, 2000a,b) were made for analysis of other crops. Problems with rice hull matrix interference were overcome by modification of the GC temperature programme.

Validation data are shown in Table 93. As an internal standard was used, recoveries in Table 93 represent calibration errors, which is why they are scattered rather randomly above and below 100%.

Table 93. Recoveries of PCA from crop samples by method PTRL 645W and its modifications. Numbers in parentheses are numbers of control samples.

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
original method								PTRL 645W, 5 June 1997
whole rice	0.005	0.005	4	121	105-141	12	<0.0015 (3)	Ruzo and Rose, 1998, GLP
rice grain		0.01	4	114	107-119	4.3		(validation)
rice grain	0.005	0.005	4	89	83-94	5.6	<0.005 (5)	Gaydosh and Puhl, 1998, GLP (residue trial; RP-98029)
rice grain	0.005	0.005	8	112	104-125	6.2	<0.005 (7)	Gaydosh *et al.*, 1997a, GLP (residue trial; RP-96012)
rice grain	0.005	0.10	12	94	88-104	6.4	<0.0015 (6)	Hathcock and Ruzo, 1998b, GLP

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
(freshly spiked aged samples)								(storage stability, RP-97026)
whole rice	0.005	0.005	2	106	105-107	-	<0.005 (2)	Gaydosh *et al.*, 1997b, GLP (processing; RP-96013)
whole rice	0.005	0.005	2	109	106-112	-	<0.005 (2)	Gaydosh, 1998b, GLP (processing; RP-95028)
polished rice	0.005	0.005 0.01	2 2	106 103	104-108 102-103	- -	<0.0015 (2)	Ruzo and Rose, 1998, GLP (validation)
white rice	0.005	0.005	2	99	98-100	-	<0.005 (2)	Gaydosh *et al.*, 1997b, GLP (processing; RP-96013)
white rice	0.005	0.005	2	103	99-107	-	<0.005 (2)	Gaydosh, 1998b, GLP (processing; RP-95028)
rice straw	0.005	0.005 0.01	2 2	115 108	109-121 101-114	- -	<0.0015 (2)	Ruzo and Rose, 1998, GLP (validation)
rice straw	0.005	0.005	6	85	60-101	4.7	<0.005 (5)	Gaydosh and Puhl, 1998, GLP (residue trial; RP-98029)
rice straw	0.005	0.005	8	101	71-116	13	<0.005 (7)	Gaydosh *et al.*, 1997a, GLP (residue trial; RP-96012)
rice straw (freshly spiked aged samples)	0.005	0.10	12	96	81-107	8.0	<0.0015 (6)	Hathcock and Ruzo, 1998b, GLP (storage stability, RP-97026)
rice straw	0.005	0.005	2	96	95-97	-	<0.005 (2)	Gaydosh *et al.*, 1997b, GLP (processing; RP-96013)
rice straw	0.005	0.005	4	92	85-100	7.9	<0.005 (2)	Gaydosh, 1998b, GLP (processing; RP-95028)
rice bran	0.005	0.005 0.01	2 2	98 97	99-98 97-98	- -	<0.0015 (2)	Ruzo and Rose, 1998, GLP (validation)
rice bran	0.005	0.005	2	100	99-100	-	<0.005 (2)	Gaydosh *et al.*, 1997b, GLP (processing; RP-96013)
rice bran	0.005	0.005	2	98	95-101	-	<0.005 (2)	Gaydosh, 1998b, GLP (processing; RP-95028)
rice bran (freshly spiked aged samples)	0.005	0.10	12	95	83-105	7.8	<0.0015-0.0015 (6)	Hathcock and Ruzo, 1998b, GLP (storage stability, RP-97026)
rice hulls	0.005	0.005 0.01	2 2	110 113	109-110 111-115	- -	<0.0015 (2)	Ruzo and Rose, 1998, GLP (validation)
rice hulls	0.005	0.005	2	104	103-104	-	<0.005 (2)	Gaydosh *et al.*, 1997b, GLP (processing; RP-96013)
rice hulls	0.005	0.005	2	103	101-105	-	<0.005 (2)	Gaydosh, 1998b, GLP (processing; RP-95028)
rice hulls (freshly spiked aged samples)	0.005	0.10	12	92	74-101	9.3	<0.0015-0.0015 (6)	Hathcock and Ruzo, 1998b, GLP (storage stability, RP-97026)
modification A								Rose, 1998b
lettuce	0.005	0.005 0.01	2 2	102 103	99-104 100-105	- -	<0.0015 (2)	Rose, 1998b, GLP (validation, RP-95018)
lettuce	0.005	0.005	26	98	76-111	6.1	<0.0015 (13)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
lettuce	0.005	0.10	11	96	92-101	2.6	<0.0015 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
turnip tops	0.005	0.005 0.01	2 2	101 100	98-103 100-100	- -	<0.0015 (2)	Rose, 1998b, GLP (validation, RP-95018)
turnip tops	0.005	0.005	24	94	71-102	7.4	<0.0015 (12)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
turnip roots	0.005	0.005 0.01	2 2	100 102	99-101 101-102	- -	<0.0015 (2)	Rose, 1998b, GLP (validation, RP-95018)
turnip roots	0.005	0.005	24	99	88-108	5.1	<0.0015 (12)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
turnip roots	0.005	0.10	12	97	88-102	5.0	<0.0015 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
wheat forage	0.005	0.005 0.01	2 2	107 108	107-107 108-108	- -	<0.0015 (1)	Rose, 1998b, GLP (validation, RP-95018)

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
wheat forage	0.005	0.005	18	101	87-115	6.9	<0.0015 (9)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
wheat grain	0.005	0.005 0.01	2 2	88 93	84-91 91-94	- -	<0.0015 (2)	Rose, 1998b, GLP (validation, RP-95018)
wheat grain	0.005	0.005	20	91	79-104	8.8	<0.0015 (10)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
wheat grain	0.005	0.10	12	97	77-104	7.0	<0.0015-0.0015 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
wheat hay	0.005	0.005	21	98	83-111	7.1	<0.0015 (11)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
wheat hay	0.005	0.10	12	99	89-104	5.0	<0.0015 (6)	Hathcock and Ruzo, 1998a, GLP (storage stability, RP-97027)
wheat straw	0.005	0.005 0.01	2 2	88 104	83-92 102-106	- -	<0.0015 (2)	Rose, 1998b, GLP (validation, RP-95018)
wheat straw	0.005	0.005	22	102	76-132	11	<0.0015-0.0038 (11)	Gaydosh, 1999d, GLP (rotational crops, RP-95018)
modification B								Nishioka *et al.*, 1997
pear	0.005	0.005 0.01	2 2	95 96	93-97 94-99	- -	<0.0015 (1)	Nishioka *et al.*, 1997, GLP (validation, RP-96025)
pear	0.005	0.005	4	121	113-127	4.9	<0.005 (2)	Gaydosh and Rose, 1999, GLP (residue trial, RP-96025)
pear	0.005	0.005	14	103	87-122	12	<0.005 (14)	Dorschner and Gaydosh, 2000 GLP, (residue trial, IR-4)
pear (freshly spiked aged samples)	0.005	0.10	10	101	79-115	11	<0.005 (5)	Nishioka *et al.*, 1997, GLP (storage stability, RP-96025)
modification C								Rose, 1999a
peach	0.005	0.005 0.01	2 2	98 100	96-101 100-101	- -	<0.0015 (1)	Rose, 1999a, GLP (validation, RP-97008)
peach	0.005	0.005	10	99	91-103	4.4	<0.005 (6)	Gaydosh, 1999b, GLP (residue trial, RP-97008)
modification D								Rose, 1999c
peach	0.005	0.005 0.01	3 3	94 107	88-104 103-113	9.3 4.8	<0.0015 (2)	Rose, 1999c, GLP (validation, RP-98001)
peach	0.005	0.005	8	82	61-98	14	<0.005 (6)	Gaydosh, 2000a, GLP (residue trial, RP-98001)
modification E								Rose, 2000a
plum	0.005	0.005 0.01	3 3	86 99	77-91 95-104	9.1 4.5	<0.0015 (2)	Rose, 2000a, GLP (validation, RP-98002)
plum	0.005	0.005	8	101	90-111	6.9	<0.005 (5)	Gaydosh, 2000b, GLP (residue trial, RP-98002)
prune	0.005	0.005 0.01	3 3	100 107	99-103 100-117	2.3 8.5	<0.0015 (2)	Rose, 2000a, GLP (validation, RP-98002)
prune	0.005	0.005	2	98	98-99	-	<0.0015 (1)	Gaydosh, 2000b, GLP (residue trial, RP-98002)
modification F								Rose, 1999b
pepper	0.005	0.005 0.01 0.05	6 6 6	98 101 98	93-104 96-106 80-117	4.3 4.2 13	<0.0015 (4)	Rose, 1999b, GLP (validation, RP-97016)
pepper	0.005	0.005	6	90	69-103	13	<0.005 (9)	Gaydosh and Puhl, 1999, GLP (residue trial, RP-97016)
pepper (freshly spiked aged samples)	0.005	0.2	10	88	73-101	12	<0.0015 (5)	Rose, 1999b, GLP (storage stability, RP-97016)
modification G								Rose, 1998a
mushrooms	0.01	0.01	2	113	113-113	-	0.0048-0.0051 (2)	Rose, 1998a, GLP (validation, RP-97004)
mushrooms	0.01	0.01	32	106	89-160	11	<0.005-0.0056 (16)	Gaydosh, 1998a, GLP (residue trial, RP-97004)
modification H								Rose, 2000b
mushrooms	0.005	0.1	12	89	78-110	13	<0.0015-0.0051 (6)	Rose, 2000b, GLP (storage stability, RP-98024)

Sample	LOQ, mg/kg	Spike, mg/kg	No.	% Rec mean.	% Rec range	% RSD$_r$	Controls mg/kg	Reference
modification I								Rose, 1999d
almond kernels	0.005	0.005 0.01	3 3	76 87	69-88 80-98	14 11	<0.0015 (2)	Rose, 1999d, GLP (validation, RP-98003)
almond kernels	0.005	0.005	6	108	101-117	6.1	<0.005 (5)	Gaydosh, 2000c, GLP (residue trial, RP-98003)
almond hulls	0.005	0.005 0.01	3 3	93 95	82-100 83-102	10 11	<0.0015-0.0015 (2)	Rose, 1999d, GLP (validation, RP-98003)
almond hulls	0.005	0.01	6	104	94-111	6.6	<0.005 (5)	Gaydosh, 2000c, GLP (residue trial, RP-98003)
modification J								Rose, 1999f
pecan kernels	0.005	0.005	10	104	97-120	7.7	<0.005 (5)	Gaydosh, 2000d, GLP (residue trial, RP-99002)
pecan hulls	0.005	0.005	8	90	75-104	12	<0.005 (5)	Gaydosh, 2000d, GLP (residue trial, RP-99002)

Ruzo and Rose (1998, GLP, fortified rice grain and rice straw with 4 mg/kg diflubenzuron or CPU. Less than 0.04% of the diflubenzuron and CPU was converted to PCA, indicating good selectivity for PCA. Recoveries from samples without internal standard were generally below 48%.

Low recoveries from rice grain and straw by ECD method CARDC-1248-PCA without internal standard are shown in Table 87. These recoveries were considered unacceptably low.

Method PTRL 645W was proposed as an enforcement method for rice. Rose (1997, GLP) reported on linearity and matrix interferences. The 4-point calibration curve for [^{12}C]PCA standards was linear (r^2=0.998) in the range 0.005-0.05 mg/l. The 4-point calibration curve for [^{12}C]PCA:[^{13}C]PCA with a fixed concentration of 0.005 mg/kg [^{13}C]PCA was linear (r^2=1.000) for the mass ratio range 0.5:1 to 10.0:1. Because of matrix interferences at m/z 323 and 126, apparent PCA levels of 0.001 mg/kg were found in rice grain and straw.

Cassidy (1998b, non-GLP) reported an independent laboratory validation. It was found that when higher residues of PCA had to be determined, the internal standard (0.005 mg/kg) was not detectable because of dilution. The internal standard was therefore added at 0.8 mg/kg at the fortification level of 0.8 mg/kg PCA. Duplicate samples of rice grain fortified at 0.005 and 0.02 mg/kg PCA gave recoveries of 82% and 78% respectively, and rice straw fortified at 0.005 and 0.8 mg/kg PCA gave 106% and 103% respectively.

Jalali and Hiler (1997, GLP) compared the results of the metabolism study by Walsh (1997) with those of Rose (1997) as shown in Table 94. The proposed PCA method showed a bias for incurred PCA levels (free plus conjugated) of -30% at 0.056 mg/kg diflubenzuron equivalents in one sample of straw.

It is unreasonable to base any estimate of bias on a single result. The time between the extraction of samples in the metabolism study and in the analytical method study was 12 months, and bias could be caused by instability in storage. With a boiling point of about 230°C, losses of free PCA could also occur by simple volatilization. Another possibility is that extraction and hydrolysis were more efficient in the reference method than in the residue method.

A confirmatory technique for rice was not proposed and the method was unpublished. Because of internal standardization, verification of derivatization efficiency for PCA is not required.

Table 94. Comparison of methods for the determination of PCA in ^{14}C-treated rice plants.

Sample	Treatment (kg ai/ha)	TRR mg/kg diflubenzuron equivalents (Walsh, 1997)	TRR mg/kg diflubenzuron equivalents (Jalali and Hiler, 1997)	Distribution in extracts and solids (Walsh, 1997)	PCA (mg/kg diflubenzuron equivalents) HPLC-LSC (Walsh, 1997)	PCA (mg/kg diflubenzuron equivalents) GC-MS (Rose, 1997)
Rice grain	0.28	0.091	0.091	extracts diflubenzuron 0.2% CPU 17%	not determined	<0.005
	1.7	0.66	0.64	extracts diflubenzuron 0.3% CPU 22% CPU conj 0.9% PCA 0.3% hydrolysis of solids: none	0.002	<0.005
Rice straw	0.28	1.0	0.88	extracts diflubenzuron 36%; CPU 26%;	not determined[1]	0.0058
	1.7	9.0	8.2	extracts diflubenzuron 42%; CPU 29%; CPU conj 2.5%; PCA 0.2%; hydrolysis of solids: CPU 10% PCA 0.4%	0.019 (extr) 0.037 (solid) total = 0.056	0.039

[1] The authors refer to a concentration of 0.019 mg/kg diflubenzuron equivalents in low-dose straw, but in the original study report (Walsh, 1997) PCA was not determined in the low-dose straw

Stability of pesticide residues in stored analytical samples

The Meeting received data on the stability of residues in plant products (grapefruit, lemons, limes, oranges, apples, pears, tomatoes, peppers, mushrooms, lettuce, turnip roots, wheat grain, wheat hay, rice commodities), animal products (chicken manure, muscle, liver, egg white and yolk, cow's milk, goat liver and milk) and soils stored frozen.

Plant products

Van Zijtveld *et al.* (1989, non-GLP) fortified ground oranges (pineapple variety) and grapefruit (red marsh) with 0.01, 0.05, 0.2, 0.5 and 1.0 mg/kg diflubenzuron. Another group of whole citrus fruits (from a greengrocer and from an untreated plot) were spiked by pipetting about 0.01 mg/kg PCA on the peel. Samples were kept frozen at –20°C for 19 weeks for diflubenzuron and 0-2 days for PCA. Samples were analysed by GC-ECD method LAI-3-86-6 for diflubenzuron and GC-ECD method RES027 for PCA.

The mean recovery of fresh spikes at 0.5 mg/kg diflubenzuron was 86% (Table 74). The diflubenzuron remaining after 19 weeks of storage (single samples) was 45%, 34%, 71%, 37% and 55% for oranges and 18%, 60%, 46%, 58% and 47% for grapefruit at 0.01, 0.05, 0.2, 0.5 and 1.0 mg/kg diflubenzuron. Results were corrected for matrix interferences (max. 0.020 mg/kg diflubenzuron) but not for concurrent method recoveries.

Because of matrix interferences up to 0.020 mg/kg diflubenzuron (Table 74), the storage results at 0.01 and 0.05 mg/kg diflubenzuron were disregarded.

Mean recoveries of fresh spikes at 0.001-0.010 mg/kg PCA were 28-41% in oranges (Table 86). The PCA remaining on oranges spiked at about 0.01 mg/kg was 35% ± 61% at day 0, 21% ±

127% at day 2 and 0% at day 5. Results were not corrected for concurrent method recoveries. Matrix interferences were negligible.

Because of low concurrent method recoveries, the storage results for PCA were disregarded.

Allan *et al.* (1997, GLP) fortified homogenized lemons and oranges with 0.1 and 1.0 mg/kg diflubenzuron. Samples kept frozen for 4 and 5 months at –10°C were analysed for diflubenzuron by HPLC method RES045. Concurrent method recoveries were not determined. The results are shown in Table 95.

Because of high recoveries (113%, 145%), the results at 0.1 mg/kg were disregarded.

Table 95 Storage stability of diflubenzuron in oranges and lemons fortified at 0.1 or 1.0 mg/kg (n = 4).

Sample	Storage time (months)	0.1 mg/kg diflubenzuron	1.0 mg/kg diflubenzuron
lemons	4	93±11%	83±4.4%
	5	113±5.1%	82±3.0%
oranges	4	145±5.5%	97±2.1%
	5	97±6.4%	88±4.0%

Rose and McIntosh (1998, GLP) fortified macerated limes with 0.5 mg/kg diflubenzuron and kept the samples frozen for 0, 1, 3 and 6 months. Samples were analysed for diflubenzuron by the GC-ECD method of Duphar (1990).

The mean recovery of fresh spikes was 92% (Table 78). The diflubenzuron remaining after 1, 32, 94 and 189 days of storage (n=2) was 93%, 106%, 73% and 80% respectively. Results were not corrected for concurrent method recovery nor for matrix interferences.

Allan and Thus (1999, GLP) fortified homogenized lemons and oranges with 0.1 and 1.0 mg/kg diflubenzuron. Samples frozen for 1 week and 4.5 months at –10°C were analysed for diflubenzuron by HPLC method RES045. Concurrent method recoveries were not determined. Because the method was hindered by serious matrix interferences at 0.1 mg/kg (resulting in recoveries between 19% and 172%), only the results at 1.0 mg/kg are shown in Table 96.

Because the (non-concurrent) method recoveries at 0.1-0.8 mg/kg were all below 70% (Table 61), the lemon results were disregarded.

Table 96 Storage stability in oranges and lemons (n=4) fortified with 1.0 mg/kg diflubenzuron.

Sample	Storage time	1.0 mg/kg diflubenzuron
lemons	1 week	98±5.7%
	4.5 months	69±12%
oranges	1 week	79±4.1%
	4.5 months	72±20%

Pouwelse (1989, GLP) fortified apples with 0.01-1.0 mg/kg diflubenzuron and kept the samples at –20°C for 1.5 months. They were analysed for diflubenzuron by HPLC method LAI 3-86-14.

Concurrent method recoveries were not determined. Diflubenzuron recoveries (single samples at each level) after 1.5 months of storage were 70% (0.01 mg/kg), 81% (0.03 mg/kg), 83% (0.05 mg/kg), 67% (0.1 mg/kg), 74% (0.5 mg/kg), and 92% (1 mg/kg).

Because of matrix interferences (up to 0.028 mg/kg, Table 56), storage results at 0.01-0.05 mg/kg were disregarded.

Thus and Allan (1995, GLP) fortified apples with 0.1 and 1.0 mg/kg diflubenzuron and kept them at –10°C for 7 weeks. They were analysed by HPLC method RES060.

Mean recoveries of fresh spikes were 82% and 99% (Table 58). Diflubenzuron recoveries after 7 weeks of storage (n=4 at each level) were 74% (0.1 mg/kg) and 97% (1 mg/kg). The results were not corrected for concurrent method recovery, nor for matrix interferences.

Nishioka *et al.* (1997, GLP) and Rose (1999e, GLP) fortified macerated pears with 0.05 mg/kg diflubenzuron, 0.1 mg/kg CPU or 0.1 mg/kg PCA. Samples frozen for 0, 1, 3, 6 or 12 months were analysed by GC-ECD method 3-86-6 for diflubenzuron, GC-ECD method LAI 3-86-9 for CPU and method PTRL 645W for PCA. Fortified control samples were extracted concurrently with the storage stability samples.

Mean recoveries of fresh spikes were 75% for diflubenzuron (Table 74), 97% for CPU (Table 83) and 101% for PCA (Table 93). The storage stability results are shown in Table 97.

Table 97 Storage stability of residues in pears (n=2 or 3) fortified with 0.05 mg/kg diflubenzuron or 0.1 mg/kg CPU or PCA.

Storage (days) diflubenzuron-CPU-PCA	diflubenzuron uncorr	diflubenzuron corr	CPU uncorr	CPU corr	PCA uncorr	PCA corr
1-2-3	76%	100%	103%	100%	96%	100%
29-31-35	74±1.2%	98%	86%	97%	40%	47%
73- 92-94	72±2.1%	104%	96±1.8%	100%	23±17%	20%
na-183-190	na	na	32±1.6%	32%	24±7.2%	21%
na-na-365	na	na	na	na	14%	14%

na: not analysed
uncorr: not corrected for method recovery or for matrix interferences
corr: corrected for concurrent method recovery and matrix interferences

Pouwelse and Van Zijtveld (1991, GLP) fortified chopped tomatoes with 0.03-1.0 mg/kg diflubenzuron and kept them at –20°C for 10 months. Samples were analysed by HPLC method LAI-3-86-2. Method recoveries of fresh spikes were 96%-98% (Table 48). Stored results were 87% (0.03 mg/kg), 94% (0.05 mg/kg), 74% (0.1 mg/kg), 90% (0.5 mg/kg) and 95% (1.0 mg/kg).

Because of matrix interferences (up to 0.018 mg/kg, Table 48), storage results for 0.03 and 0.05 mg/kg were disregarded.

Rose (1999b, GLP) fortified macerated peppers with 0.5 mg/kg diflubenzuron or 0.1 mg/kg CPU or PCA and kept samples frozen for 0, 1, 3, 6 or 12 months before analysis by separate methods for each analyte as above. Fortified control samples were extracted concurrently with the storage stability samples.

Mean recoveries of fresh spikes at 0.2 mg/kg were 92% for diflubenzuron (Table 74), 88% for CPU (Table 83) and 88% for PCA (Table 93). The storage stability results are shown in Table 98.

Table 98. Storage stability of residues in peppers (n=2 or 3) fortified with 0.5 mg/kg diflubenzuron or 0.1 mg/kg CPU or PCA.

Storage (days) diflubenzuron-CPU-PCA	diflubenzuron uncorr	diflubenzuron corr	CPU uncorr	CPU corr	PCA uncorr	PCA corr
0-0-0	72%	100%	91%	100%	85%	100%
23-31-32	135%	106%	64%	90%	56±5.5%	59%
86-89-90	70±8.4%	91%	77±9.8%	73%	48±7.2%	48%

Storage (days) diflubenzuron-CPU-PCA	diflubenzuron uncorr	diflubenzuron corr	CPU uncorr	CPU corr	PCA uncorr	PCA corr
174-186-183	79±3.7%	100%	65±1.8%	75%	31±1.9%	41%
369-367-366	79±6.3%	94%	55±2.8%	72%	19±5.3%	22%

na: not analysed
uncorr: not corrected for method recovery or matrix interferences
corr: corrected for concurrent method recovery but not for matrix interferences

Rose (2000b, GLP) fortified mushrooms with 0.1 mg/kg diflubenzuron, CPU or PCA and kept samples below 0°C for 0, 1, 3, 6, 12, 18 or 19 months. The samples were analysed by HPLC method LAI 3-86-13 for diflubenzuron, GC-ECD method LAI 3-86-9 for CPU and method PTRL 645W for PCA.

Mean recoveries of fresh spikes at 0.1 mg/kg were 95% for diflubenzuron (Table 53), 90% for CPU (Table 83) and 89% for PCA (Table 93). The storage stability results are shown in Table 99; they were not corrected for method recoveries.

Table 99. Storage stability of residues in mushrooms fortified with 0.1 mg/kg diflubenzuron, CPU or PCA (means of duplicate analyses).

Storage (days) diflubenzuron-CPU-PCA	% remaining		
	diflubenzuron	CPU	PCA
0 – 0 - 0	104	77	79
31 – 29 – 30	104	79	14
91 – 104 - 97	104	106	18
192 – 180 - 182	85	86	13
361 – 369 – 364	89	77	28
536 – 540 - 540	68	90	27
na –575 – na	na	90	na

na: not analysed

Hathcock and Ruzo (1998a, GLP) fortified lettuce, turnip roots, wheat grain and hay with 0.1 mg/kg diflubenzuron, CPU or PCA and kept samples below 0°C for 0, 1, 3, 6, 9 or 12 months before analysis by methods 3-86-6 for diflubenzuron, LAI 3-86-9 for CPU and 645W for PCA.

Mean recoveries of fresh spikes at 0.1 mg/kg were 77%-90% for diflubenzuron (Table 74), 82%-100% for CPU (Table 83) and 96%-99% for PCA (Table 93). The storage stability results are shown in Table 100.

Because modifications were made to the analytical methods for diflubenzuron and CPU during the stability study to accommodate apparent matrix changes over time (after 3-6 months in lettuce and wheat grain), only the results for 1 month storage are considered.

Table 100. Storage stability of samples fortified with 0.1 mg/kg diflubenzuron, CPU, or PCA (means of duplicate analyses).

Sample	Storage (days) diflubenzuron-CPU-PCA	% remaining				
		diflubenzuron	diflubenzuron[1]	CPU	CPU[1]	PCA
lettuce	0 –0 – 0	87	-	88	-	95
	32 –28 – 29	103	137	107	98	43
	112 – 93 – 102	62	58	73	69	27
	206 – 191 – 182	60	135	92	73	30
	266 – 271 – 273	87	114	66	93	32
	380 – 341 – 358	88	117	80	78	25
turnip roots	0 – 0 – 0	79	-	87	-	100

Sample	Storage (days) diflubenzuron-CPU-PCA	% remaining				
		diflubenzuron	diflubenzuron[1]	CPU	CPU[1]	PCA
	32 – 28 – 29	98	96	93	108	78
	112 – 93 – 91	43	52	77	77	72
	206 – 183 – 182	64	82	89	97	71
	276 – 271 – 274	79	106	86	93	66
	378 – 341 – 358	77	98	112	109	63
wheat grain	0 – 0 – 0	90	-	78	-	99
	31 – 34 – 30	157	134	68	96	68
	135 – 89 – 93	48	39	31	31	46
	206 – 187 – 178	59	99	32	42	50
	276 – 278 – 276	66	91	50	56	50
	382 – 391 – 351	68	89	42	51	46
wheat hay	0 - 0 – 0	102	-	83	-	102
	31 – 28 – 30	149	122	124	140	69
	135 –96 – 93	62	66	97	91	54
	203 – 187 – 178	52	69	43	55	59
	276 – 293 – 276	72	101	107	92	52
	394 –356 – 351	49	73	69	85	52

[1] corrected for method recovery

Hathcock and Ruzo (1998b, GLP) stored rice grain, bran, straw and hulls fortified with 0.1 mg/kg diflubenzuron, CPU or PCA at -12 to -25°C for 0, 1, 3, 6, 9 or 12 months and analysed them as above.

Mean recoveries of fresh spikes at 0.1 mg/kg were 69%-84% for diflubenzuron (Table 74), 75%-105% for CPU (Table 83) and 92%-96% for PCA (Table 93). The storage stability results are shown in Table 101.

Because modifications were made to the analytical methods for diflubenzuron and CPU during the stability study to accommodate apparent matrix changes over time (after 3-6 months in lettuce and wheat grain), only the results for 1 month storage are considered.

Table 101. Storage stability of diflubenzuron, CPU, and PCA (n=2) in rice samples fortified at 0.1 mg/kg.

Sample	Storage (days) diflubenzuron-CPU-PCA	diflubenzuron	diflubenzuron[1]	CPU	CPU[1]	PCA
grain	0 – 0 – 0	79	-	85	-	91
	30 – 28 – 27	93	98	74	90	40
	91 – 123 – 87	57	71	47	61	27
	224 – 183 – 175	43	82	44	61	29
	287 – 283 – 273	51	56	70	97	23
	345 – 343 – 336	59	82	55	71	23
bran	0 – 0 – 0	101	-	101	-	96
	28 – 29 – 27	106	100	102	113	52
	89 – 123 – 87	59	74	38	44	36
	228 – 186 – 175	11	96	73	74	34
	313 – 290 – 273	78	114	81	76	35
	365 – 338 – 336	55	106	103	103	31
straw	0 - 0 – 0	103	-	108	-	101
	41 – 29 – 27	93	104	99	114	67
	90 – 92 – 90	53	63	89	102	49
	222 – 186 – 175	45	55	99	80	40
	285 – 295 – 273	76	86	127	116	42
	355 – 343 – 336	57	125	100	85	37
hulls	0 – 0 - 0	103	-	72	-	100
	34 – 42 – 27	116	118	81	109	65
	92 – 88 – 90	59	67	44	51	48

Sample	Storage (days) diflubenzuron-CPU-PCA	diflubenzuron	diflubenzuron[1]	CPU	CPU[1]	PCA
	230 – 190 – 175	52	68	33	44	30
	295 – 290 – 273	40	57	47	51	42
	350 – 346 – 336	61	96	42	73	30

[1] corrected for method recovery

Animal products

Buisman and Verhaar (1987a, non-GLP) reported the storage stability of diflubenzuron in egg white and yolk using HPLC method LAI 3-86-17. Whites were fortified with 0.05 and 0.1 mg/kg diflubenzuron and yolks with 0.05 and 0.5 mg/kg and stored at -20°C for 1 year. Mean recoveries of fresh spikes were 67% for yolks and 76% for whites (Table 65). Storage stability data with and without correction for method recoveries are shown in Table 102.

Because the mean method recovery from yolks was below 70%, the storage results for yolks were disregarded.

Table 102 Storage stability of diflubenzuron in fortified egg whites and yolks (1 year, -20°C).

Sample	spike (diflubenzuron, mg/kg)	storage stability (n=5, mean±RSD_r)	storage stability (corrected for method recovery)
egg whites	0.05	71% ± 3.8%	93% ± 3.8%
	0.1	74% ± 3.3%	98% ± 3.3%
egg yolks	0.05	64% ± 6.8%	95% ± 6.8%
	0.5	78% ± 2.3%	116% ± 2.3%

Buisman and Snijders (1987b, non-GLP) reported the storage stability of diflubenzuron in chicken manure using method LAI 3-86-2. Chicken manure obtained from a feed-through study with 7.5 mg/kg diflubenzuron in the feed was stored for 4 months at –20°C before commencement of the stability experiment, in which the manure was stored for 0, 3, 6 and 12 months at ambient temperatures in the dark. Method recoveries were determined by fortification of control samples on the day of extraction of the storage stability samples.

Maximum matrix interferences were equivalent to 0.043 mg/kg diflubenzuron (n=4). Method recoveries of fresh spikes were 74%-115% at levels between 2.1 and 6.7 mg/kg diflubenzuron (single samples, Table 48). The storage results are shown in Table 103.

Because the experiment started after a 4-month storage period at –20°C and the initial concentration was not reported, the study was disregarded.

Table 103. Storage stability of diflubenzuron in chicken manure (ambient temperatures) from a feed-through study.

Storage (month's)	diflubenzuron mg/kg[2] (n=3, mean ± RSD_r)	storage stability
0[1]	4.2±7.5%	Taken as 100%
3	4.1±7.7%	97%
6	3.6±6.0%	86%
12	1.7±24%	40%

[1] Sample was stored for 4 months at –20°C

[2] dry weight at time 0 was 34%

Buisman and Verhaar (1987b,c, GLP) reported the storage stability of diflubenzuron in cow's milk using a combination of methods LAI 3-86-19 and LAI 3-86-23 (full description not available to the reviewer). Control samples were fortified with 0.05 and 0.1 mg/kg diflubenzuron and stored at -20°C for 1 year.

Maximum matrix interferences were 0.002 mg/kg diflubenzuron (n=4). Recoveries from freshly spiked and stored samples are shown in Table 104.

Table 104. Storage stability of diflubenzuron in fortified cow's milk (1 year, -20°C).

spike (diflubenzuron, mg/kg)	method recovery (n=4, mean±RSD_r)	% remaining (n=5)
0.05	90% ± 9.7%	82 ± 11
0.1	90% ± 2.1%	85 ± 2.6

Timmerman *et al.* (1992a, GLP) summarized the results of summaries of the stability of diflubenzuron, CPU, PCA, and PCAA in spiked goat liver and milk stored for 22 months at or below –10°C (Dijksman *et al.*, 1990, Timmerman *et al.*, 1992b,c). Diflubenzuron, CPU and PCAA were determined by HPLC method D (Table 68) and PCA by a modification of GC-ECD method K (Table 88).

Mean recoveries of fresh spikes of PCA from milk at 0.0025 mg/kg were 82% (n=2). Mean recoveries of fresh spikes of diflubenzuron, CPU and PCAA from milk and liver are shown in Table 68, and of PCA from milk and liver homogenate in Table 88. Storage stability results are shown in Table 105.

Table 105. Storage stability of residues in goat liver and milk spiked with mixtures of diflubenzuron, CPU, PCA and PCAA (22 months at -10°C).

Mixture	Compound	Spike (mg/kg)	Storage stability milk (mean ± RSD_r)	Storage stability liver (mean ± RSD_r)
1	diflubenzuron	0.020	127% ± 33% (n=3)	79% ± 98% (n=4)
	CPU	0.020	110% ± 48% (n=3)	143% (n=2)[1]
	PCA	0.002	65% ± 18% (n=6)	130% ± 30% (n=3)
	PCAA	0.020	98% ± 36% (n=3)	101% ± 56% (n=4)
2	diflubenzuron	0.080	75% ± 17% (n=3)	107% ± 58% (n=4)
	CPU	0.080	88% ± 26% (n=3)	107% ± 23% (n=4)
	PCA	0.008	na	96% ± 36% (n=3)
	PCAA	0.080	88% ± 28% (n=3)	81% ± 12% (n=4)
3	diflubenzuron	1.5	60% ± 11% (n=5)	na
	CPU	1.5	66% ± 10% (n=3)	85% ± 16% (n=4)
	PCA	0.14	na	116% ± 24% (n=3)
	PCAA	1.5	82% ± 18% (n=3)	91% ± 29% (n=4)

na: not analysed

[1] 2 runs discarded (no signal detected)

Cnubben *et al.* (1996, GLP) reported the storage stability of diflubenzuron, CPU, PCA and PCAA in homogenates of chicken liver, thigh muscle, and egg yolk spiked with a mixture of ^{14}C-labelled diflubenzuron (0.04 mg/kg), CPU (0.04 mg/kg), PCA (0.02 mg/kg) and PCAA (0.04 mg/kg) and stored for 10 months at –195°C, 12 months at –80°C or 15 months at –20°C. Samples mixed with DMSO and silica gel were applied to a pre-column above a C-8 HPLC column. Diflubenzuron and the metabolites were separated using an elution gradient with UV (254 nm) and on-line radiochemical detection.

The HPLC system gave sufficient separation of all four compounds. The recovery of total radioactivity from egg yolk homogenates spiked on the day of extraction with diflubenzuron, CPU, PCA and PCAA was 95%. The spike levels and the recovery of individual compounds were not reported. The results are shown in Table 106.

Table 106. Storage stability of residues in chicken tissues and egg yolks spiked with a mixture of diflubenzuron, CPU, PCA and PCAA.

Sample	Compound	Spike, (mg/kg)	10 months -195°C	12 months –80°C	15 months -20°C
egg yolk	diflubenzuron	0.040	90%	82%	82%
	CPU	0.040	85%	80%	80%
	PCA	0.020	85%	90%	90%
	PCAA	0.040	90%	85%	85%
chicken liver	diflubenzuron	0.040	98%	80%	80%
	CPU	0.040	92%	82%	132%
	PCA	0.020	35%	80%	25%
	PCAA	0.040	142%	80%	58%
chicken thigh muscle	diflubenzuron	0.040	88%	92%	90%
	CPU	0.040	90%	90%	78%
	PCA	0.020	0%	0%	25%
	PCAA	0.040	170%	168%	120%

Soil

Kramer (1990,1991, GLP) reported the storage stability of diflubenzuron, CPU and DFBA in soil spiked in duplicate with 0.5 and 5.0 mg/kg diflubenzuron, CPU or DFBA and stored at –20°C. Samples were analysed at intervals by HPLC method RES026 for diflubenzuron, GC-ECD method RES025 for CPU and GC-ECD method RES031 for DFBA.

Results were not corrected for concurrent method recoveries (83%-112% and 79%-119%) or matrix interferences (maximum 0.031 mg/kg for diflubenzuron, <0.01 mg/kg for CPU and DFBA) (Tables 107 and 108).

Table 107. Storage stability at –20°C of diflubenzuron, CPU and DFBA in spiked soil (Kramer, 1990).

Storage days	% remaining					
	diflubenzuron 0.5 mg/kg	diflubenzuron 5 mg/kg	CPU 0.5 mg/kg	CPU 5 mg/kg	DFBA 0.5 mg/kg	DFBA 5 mg/kg
0	82-97	74-96	87-87[1]	99-100[1]	98-100	86-88
30	91-92	89-95	77-79	114-118	85-100	80-90
60	74-74	94-106	73-77	97-101	88-102	85-96
90	72-81	84-105	73-79	77-81	75-82	86-102
120	85-96	80-81	87-95	124-132	79-86[2]	73-82
180	82-88	85-108	79-84	113-135	75-96	85-99
360	82-87	42-68[2]	69-71[2]	84-102[2]	78-83	81-86
436	74-74	54-67	na	na	na	na

na: not analysed

[1] results after reanalysis (first analysis rejected because of low concurrent recovery)

[2] results after reanalysis (first analysis rejected because of low storage results)

Table 108. Storage stability at –20°C of diflubenzuron, CPU, and DFBA in spiked soil (Kramer, 1991).

Storage days	% remaining					
	diflubenzuron 0.5 mg/kg	diflubenzuron 5 mg/kg	CPU 0.5 mg/kg	CPU 5 mg/kg	DFBA 0.5 mg/kg	DFBA 5 mg/kg
0	90-123	94-95	96-98	96-101	111-118	108-109
30	83-88	96-96	75-75[1]	87-92[1]	100-109	98-102

Storage days	% remaining					
	diflubenzuron 0.5 mg/kg	diflubenzuron 5 mg/kg	CPU 0.5 mg/kg	CPU 5 mg/kg	DFBA 0.5 mg/kg	DFBA 5 mg/kg
60	75-87	68-93	77-87	116-117	94-97	87-98
90	88-101	67-89	93-94	106-106	82-109	99-102
120	77-92	81-84	85-87	113-120	88-93	89-99
180-194	95-110	124-124	75-85	104-126	94-104	106-107
360	87-95	86-101	74-80	84-88	81-84	77-79
475-483	92-108	90-98	64-68	77-83	99-105	96-97

[1] results after reanalysis (first analysis rejected because of either low or high storage results)

In a third experiment (Kramer, 1992, GLP) soil spiked in duplicate with 0.5 mg/kg diflubenzuron, CPU or DFBA and stored at –20°C was treated identically.

Samples were not corrected for concurrent method recoveries (66%-107%) or matrix interferences (<0.01 mg/kg). The results are shown in Table 109.

Table 109. Storage stability at –20°C of diflubenzuron, CPU, and DFBA in spiked soil (Kramer, 1992).

Storage days	% remaining		
	diflubenzuron 0.5 mg/kg	CPU 0.5 mg/kg	DFBA 0.5 mg/kg
0	88-96	73-81	101-110
30	97-109	76-88	120-121
90	97-108	75-77	105-106
180	86-92	83-87	111-123
400	97-100	91-96	113-119

Ver Hey (1991a,b, GLP) reported the storage stability of diflubenzuron, CPU and DFBA in soil spiked with 0.5 mg/kg diflubenzuron or 0.1 mg/kg CPU or DFBA and stored at –20°C. Samples were analysed as above.

Results for diflubenzuron and DFBA were calculated without correction for concurrent method recoveries (70%-127%) or matrix interferences (<0.01 mg/kg). Results for CPU were calculated with and without correction for concurrent method recoveries.

Table 110. Storage stability (mean and RSD_r) at –20°C of diflubenzuron, CPU, and DFBA in spiked soil (Ver Hey, 1991a).

Storage days	diflubenzuron 0.5 mg/kg	CPU 0.1 mg/kg	CPU[1], 0.1 mg/kg corrected	DFBA 0.1 mg/kg
0	86±11%	58±6.4%	99%	82±17%
221-224	94±17%	90±6.8%	99%	102±14%
466-469	87±17%	63±8.0%	102%	102±9.0%

[1] corrected for concurrent method recoveries (59% at day 0, 91% at days 221-224, 62% at days 466-469)

Table 111. Storage stability (mean and RSD_r) at –20°C of diflubenzuron, CPU, and DFBA in spiked soil (Ver Hey, 1991b).

Storage days	diflubenzuron 0.5 mg/kg	CPU 0.1 mg/kg	CPU[1], 0.1 mg/kg corrected	DFBA 0.1 mg/kg
0	86±16%	59±28%	100%	95±9.7%
228	90±5.3%	72±26%	71%	99±13%
456	76±7.8%	64±25%	105%	116±9.6%

[1] corrected for concurrent method recoveries (59% at day 0, 101% at day 228, 61% at day 456)

Definition of the residue

Diflubenzuron was rapidly excreted by farm animals. In ruminant and pig muscle and fat, radioactive residues were very low and could not be characterized. In chicken muscle, about 70% of the residue was the parent and in chicken fat 99%. The liver of all farm animals except pigs contained the parent compound as one of the main residues. Kidneys of ruminants were not analysed; chicken kidney contained the parent as one of the main residues. The nature of the residue in milk is unclear; in one study 43% of the TRR was found to be the parent and in two others it was not detected. No major metabolite was identified in milk. In chicken eggs, a large part of the residue was the parent, and almost all the residue was in the yolk.

The Meeting agreed that the parent compound is a suitable marker molecule for enforcement in animal commodities and is also the compound of interest for dietary risk assessment.

The log K_{ow} of diflubenzuron is 3.89. Taking into account results from trials of direct animal treatment and farm animal feeding studies, the Meeting concluded that diflubenzuron should be classified as fat-soluble.

In plants, diflubenzuron is a surface residue when applied to the aerial parts of the plant. The compound is not degraded or translocated and can easily be washed off. Therefore in general diflubenzuron *per se* is the residue of interest both for enforcement and for dietary risk assessment.

However, in soil and water diflubenzuron is degraded to DFBA and CPU, which can be taken up by the plants. So for crops which grow in treated soil (mushrooms) or in flooded areas (rice) these metabolites are present in larger quantities than the parent. Metabolism studies showed that the residue in rice grain consists mainly of CPU, and in rice straw of both CPU and diflubenzuron. In mushrooms DFBA and CPU are the main metabolites, and the parent is mainly detected in the first flush.

Metabolism studies on rice and the supervised residue field trials in the USA available to the Meeting show that at the currently registered US maximum dose rates, CPU levels are below the reported LOQ of 0.001 mg/kg in rice grain. In mushrooms, DFBA is the main residue, although the amount varies widely among studies. Taking into account that DFBA is not a residue of particular toxicological concern, that the world-wide intake of mushrooms is quite low, and further that analytical methods for diflubenzuron, DFBA and CPU are quite laborious, the Meeting concluded that the definition of the residue for compliance with MRLs and for estimation of dietary intake should be diflubenzuron, both for plant and animal commodities. The residue is fat-soluble.

USE PATTERN

Diflubenzuron is effective against a wide variety of insect pests, predominantly from the families Lepidoptera (caterpillars) and Diptera (flies). It is useful in controlling worms (e.g. fall webworm, armyworm), weevils (e.g. rice water weevil, cotton boll weevil), beetles (e.g. oat leaf beetle, Colorado potato beetle), Psylla species, moths (e.g. codling moth, winter moth), leaf-feeding larvae, leaf miners, rust mites, larvae of sciarid and phorid flies, maize stalk borers, tortrix, earwigs, cabbage white, leafrollers, grasshoppers, aphids, and fruit flies.

Diflubenzuron is registered in many countries for the control of insect pests on fruit, vegetables, cereals, oilseed and forage crops. It is also used for the control of salmon louse

(*Lepeophteirus salmonis*) and sea louse (*Caligus elongatus*) in salmon-rearing facilities, for the control of lice and flies (*Bovicola limbatus, Bovicola ovis, Lucilia cuprina*) on sheep, goats and cattle and as a feed-through product against flies (Diptera) in pig sties and poultry housing. The information available to the Meeting on registered uses is summarized in Tables 112–116.

Forests are included in these tables because some European countries (Germany, Finland, Lithuania) defined pre-harvest intervals or MRLs for wild berries, wild mushrooms and medicinal herbs.

The manufacturer provided the information on GAP both in summarized form and as original labels. When original labels were not available this is indicated by an asterisk in the Table. Where information on the label differs from that in the summary, the information on the label is given in bold. Information on GAP for Australia (livestock), Germany (food crops) and The Netherlands (food crops) was also given by the national governments in a summarized form; this is indicated by @ in the Table.

Diflubenzuron is also registered for the control of flies in animal housing (cattle, pig, poultry) and manure heaps by topical application to breeding sites, of anchor worms (*Lernaea cyprinacea*) in ornamental fish ponds, of fleas on mink and of mosquitoes in sewage, mosquito breeding sites, marshes and water (urban centres, seaside septic tanks, road drains, open sewers, ornamental pools, stagnant or semi-stagnant water, irrigation or slow-flowing water in ditches and canals). Further registered uses are on ornamentals, non-fruit trees and orchards, tree nurseries, public greens, refuse heaps, soil casing, soil compost and non-crop areas.

Table 112. Registered pre-harvest uses of spray applications of diflubenzuron on fruits and nut trees in the field.

Crop	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
			Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Apple	Belarus *	WP 250	spray	0.12-0.25	ns	1-2	30
Apple	Belgium	SC 480; WP 250	ns	ns	0.01-0.03	ns	14
Apple	Bulgaria	WP 250	ns	ns	0.01	ns	30
Apple	China	WP 250	spray	ns	0.012-0.025	ns	ns
Apple	Croatia	SC 480	spray	ns	0.01	2 (14-21)	21
Apple	Cyprus	WP 250	ns	ns	**0.0075-0.01**	ns (28)	30
Apple	Cyprus	SC 480	ns	ns	0.01	ns (28)	14
Apple	Czech Republic	SC 480	spray	0.072-0.12	0.0072-0.012	ns	28
Apple	Denmark	WP 250	spray	0.15	ns	ns	14
Apple	Finland	SC 480	spray	0.14-0.36	**0.03-0.12**	ns	28
Apple	Finland *	WP 250	ns	0.15-0.38	ns	ns	28
Apple	France	SC 150; WP 250	ns	ns	0.01	ns	15
Apple	Germany * @	WG 800	spray	0.18-0.30	0.012-0.02	1-4 (14-21)	28
Apple	Germany * @	WG 800	LV spray	0.18-0.30	0.06-0.10	1-4 (14-21)	28
Apple	Greece	WP 250	ns	ns	0.01-0.015	ns	30
Apple	Hungary	WP 250	ns	0.075-0.12[1]	**0.005-0.012**	ns	14
Apple	Ireland	SC 240; SC480	ns	0.15	0.03	2 (21)	14
Apple	Israel *	WP 250	ns	ns	0.012	ns	ns
Apple	Italy	40[2]; 33[3]	ns	ns	**0.006-0.012**	**2 (21)**	45
Apple	Italy	WP 050; WP 250	ns	ns	0.01-0.02	ns	45
Apple	Jordan *	WP 250	ns	0.1	0.01	ns	14
Apple	Kazakhstan	SC 480	ns	0.048	ns	ns	ns
Apple	Lithuania	WP 250	spray	0.25-0.50	ns	ns	30
Apple	Macedonia	SC 480	ns	ns	0.01	ns	ns
Apple	Macedonia	WP 250	ns	ns	**0.012-0.02**	ns	14
Apple	Moldova	WP 250	ns	0.12	ns	ns	30
Apple	Morocco *	WP 250	ns	ns	0.01	ns	45

Crop	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
			Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Apple	Netherlands @	SC 480; WP 250	***spray***	***0.096-0.30***	***0.0096-0.2***	**1-2**	14
Apple	Pakistan	WP 250	ns	ns	0.01	**ns**	14
Apple	Poland	WP 250	ns	0.10-0.30	**0.0076-0.06**	ns	14
Apple	Portugal	WP 250	ns	ns	0.01	ns	14
Apple	Romania	SC 480	ns	ns	0.01	ns	14
Apple	Romania *	WP 250	ns	ns	0.0075-0.075	ns	14
Apple	Russia	WP 250	**spray**	**0.038-0.30**	**0.0025-0.030**	1	30
Apple	Slovakia	SC 480	ns	**0.096-0.12**	**0.0048-0.012**	ns	28
Apple	Slovenia	SC 480	ns	ns	0.01	3 (14-21)	14
Apple	Sweden	SC 480	ns	ns	0.01-0.02	ns	28
Apple	Turkey	WP 250	spray, ground or air	ns	0.01	3-4 (28)	14
Apple	UK	SC 480; WP250	ns	0.15	0.03	1 pre-, 2 post-blossom	14
Apple	Yugoslavia *	SC 480	spray	ns	0.01	3(14-21)	14
Apple	Yugoslavia *	WP 250	spray	ns	0.02	3	14
Apricot	Greece	WP 250	ns	ns	0.02-0.038	ns	30
Blackcurrants	Ireland	SC 240; SC 480	ns	0.15	0.03	2	14
Blackcurrants	UK	SC 480; WP 250	ns	0.15	0.0075	2	14
Cherries	Hungary	WP 250	ns	0.075-0.12	**0.005-0.012**	ns	14
Citrus	Brazil *	WP 250	spray	0.125	ns	1-2	30
Citrus	China	WP 250	spray	ns	0.0062-0.012	ns	ns
Citrus	Greece	WP 250	ns	ns	0.038	ns	30
Citrus	Guatemala	WP 250	ns	0.38	ns	ns	8
Citrus	Jordan *	WP 250	ns	0.1-0.15	0.01-0.015	ns	14
Citrus	Morocco *	WP 250	ns	ns	0.01	ns	45
Citrus	Portugal	WP 250	ns	ns	0.015[10]	ns	21
Citrus	Saudi Arabia *	WP 250	ns	0.12	ns	ns	14
Citrus	Spain	WP 250	**spray**	ns	0.01-0.015[11]	ns	30
Citrus	United Arab Emirates	WP 250	ns	ns	0.01-0.02	ns	3
Citrus	Uruguay	SC 480	spray	0.29-0.34	0.014	ns	15
Citrus	Uruguay	WP 250	spray	0.30-0.35	0.0088-0.015	ns	15
Date	Algeria *	OF 450	ns	0.068	1.4[4]	ns	ns
Fruit	Austria	WP 250	**spray**	ns	0.01[5]	ns	28
Fruit	Ireland	WP 250	**spray**	ns	0.01[6]-0.02	ns **(28)**	ns
Fruit	Norway	SC 480	**spray**	**0.14-0.24**	0.01-0.014	**1 (14-28)**	28
Fruit	Saudi Arabia *	WP 250	ns	ns	0.01-0.02	ns	28
Fruit	Spain	WP 250	**spray**	ns	0.01-0.015	ns	30
Grape	Czech Republic	SC 480	spray	0.1	0.01	ns	28
Grape	Slovakia	SC 480	ns	0.1	ns	ns	28
Grapefruit	USA	WP 250	spray	0.35	0.0037-0.075	1-3 (90)	21
Grapefruit	USA	WP 250	aerial spray	0.35	0.19-0.75	1-3 (90)	21
Hazelnut	Turkey	WP 250	spray, ground or air	ns	0.005	ns	14
Nashi pear	France	SC 150	ns	ns	0.01	ns	15
Nectarine	Hungary	WP 250	ns	0.075-0.12	**0.005-0.012**	ns	15
Olive	Greece	WP 250	ns	ns	0.01-0.02	ns	30
Orange	USA	WP 250	spray	0.35	0.0037-0.075	1-3 (90)	21
Orange	USA	WP 250	aerial spray	0.35	0.19-0.75	1-3 (90)	21
Peach	Bulgaria	WP 250	ns	ns	0.01	ns	30
Peach	Greece	WP 250	ns	ns	0.01-0.02	ns	30
Peach	Hungary	WP 250	ns	0.075-0.12	**0.005-0.012**	ns	15
Peach	Jordan *	WP 250	ns	ns	0.01-0.02	ns	14
Peach	Romania *	WP 250	ns	ns	0.0075	ns	14
Peach	Turkey	WP 250	spray, ground or air	ns	0.01	ns	14
Peach	Uruguay	WP 250; SC 480	spray	ns	0.02	ns	ns
Pear	Belgium	WP 250; SC 480	ns	ns	0.01-0.03	ns	14

Crop	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
			Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Pear	Bulgaria	WP 250	ns	ns	0.01	ns	30
Pear	Croatia	SC 480	ns	ns	**0.0096-0.012^{5}**	2 (14-21)	21
Pear	Cyprus	WP 250	ns	ns	**0.0075-0.01**	ns (28)	30
Pear	Cyprus	SC 480	ns	ns	0.01	ns (28)	14
Pear	Denmark	WP 250	spray	0.15^{12}	**0.03**	ns	14
Pear	France	WP 250; SC 150	ns	ns	0.01	ns	15
Pear	Germany, *, @	WG 800	spray	0.18-0.30	0.012-0.02	1-4 (14-21)	28
Pear	Germany, *, @	WG 800	LV spray	0.18-0.30	0.06-0.10	1-4 (14-21)	28
Pear	Greece	WP 250	ns	ns	**0.01-0.03**	ns	30
Pear	Hungary	WP 250	ns	0.075-0.12^{1}	**0.005-0.012**	ns	14
Pear	Ireland	SC 240; SC 480	ns	0.144	**0.015^{7}-0.03**	2 (21)	14
Pear	Italy	40^{2}; 33^{3}	ns	ns	**0.006-0.012**	**2 (21)**	45
Pear	Italy	WP 050; WP 250	ns	ns	0.01-0.02	ns	45
Pear	Jordan *	WP 250	ns	0.1-0.2	0.01^{8}-0.02	ns	14
Pear	Macedonia	SC 480	ns	ns	0.0096-0.012^{5}	ns	ns
Pear	Macedonia	WP 250	ns	ns	**0.012-0.02**	ns	21
Pear	Morocco *	WP 250	ns	ns	0.01	ns	45
Pear	Netherlands @	SC 480 WP 250	***spray***	***0.096-0.24***	***0.0096^{5}-0.20***	**1-2**	14
Pear	Poland	WP 250	ns	0.1-0.3	**0.0075-0.06**	ns	14
Pear	Portugal	WP 250	ns	ns	0.01	ns	14
Pear	Romania *	WP 250	ns	ns	0.0075-0.075	ns	14
Pear	Slovenia	SC 480	ns	ns	0.0096-0.012^{5}	3 (14-21)	21
Pear	Sweden	SC 480	ns	ns	0.019-0.029	ns	28
Pear	Turkey	WP 250; SC 480	spray, ground or air	ns	0.01^{9}	ns	14
Pear	UK	WP 250; SC 480	ns	0.15	0.015^{7}-0.03	1 pre-, 2 post-blossom	14
Pear	Uruguay	WP 250; SC 480	spray	ns	0.01	ns	15
Pear	Yugoslavia *	SC 480	ns	ns	0.012^{5}	3 (14-21)	21
Pear	Yugoslavia *	WP 250	ns	ns	0.02	3	21
Pistachio	Turkey	WP 250	spray, ground or air	ns	0.005	ns	14
Plum	Bulgaria	WP 250	ns	ns	0.012	ns	30
Plum	Greece	WP 250	ns	ns	0.02-0.038	ns	30
Plum	Hungary	WP 250	ns	0.075-0.12	**0.005-0.012**	ns	18
Plum	Ireland	SC 240; SC 480	ns	0.15	0.03	2 (21)	14
Plum	Poland	WP 250	ns	0.1-0.3	**0.0075-0.06**	ns	14
Plum	Romania *	WP 250	ns	ns	0.0075	ns	14
Plum	Slovakia	SC 480	ns	**0.12**	0.012	ns	28
Plum	UK	WP 250; SC 480	ns	0.15	0.03	2	14
Pome fruit	Albania *	SC 480	spray	ns	0.096	ns	14
Pome fruit	Algeria	WP 250	ns	ns	0.010	ns	ns
Pome fruit	Guatemala	WP 250	ns	0.02-0.04	0.01-0.02	ns	8
Pome fruit	Iran *	WP 250	ns	0.1	ns	ns	ns
Pome fruit	Switzerland	SC 480	ns	**0.14-0.19**	0.01	2	21
Pome fruit	Switzerland	WP 250	ns	ns	0.01	ns	21
Pome fruit	Syria *	WP 250	ns	ns	0.01-0.02	ns	ns
Pome fruit	United Arab Emirates	WP 250	ns	ns	0.01-0.02	ns	3
Quince	Cyprus	WP 250	ns	ns	**0.0075-0.01**	ns	30
Quince	Cyprus	SC 480	ns	ns	0.01	ns (28)	14

Crop	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
			Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Quince	France	SC 150	ns	ns	**0.01**	ns	15
Quince	Greece	WP 250	ns	ns	0.010-0.015	ns	30
Raspberry	Switzerland	SC 480	ns	**0.14-0.12**	0.01	2	21
Raspberry	Switzerland	WP 250	ns	ns	0.01	ns	21
Sour cherry	Hungary	WP 250	ns	0.075-0.12	**0.005-0.012**	ns	14
Stone fruit	Poland	WP 250	ns	0.10-0.30	ns	ns	14
Stone fruit	Switzerland	SC 480	ns	**0.14-0.19**	0.01	2	21
Stone fruit	Switzerland	WP 250	ns	ns	0.01	ns	21
Tangerine	USA	WP 250	spray	0.35	0.0037-0.075	1-3 (90)	21
Tangerine	USA	WP 250	aerial spray	0.35	0.19-0.75	1-3 (90)	21
Tree nuts	France	SC 150	ns	ns	0.01	ns	28
Walnut	Portugal	WP 250	ns	ns	0.01	ns	14

ns: not specified

* no printed label or registration certificates available (confirmed by applicant)

@ information on GAP provided by the national government in summarized form

bold: difference between label/registration certificate and applicant's summary. Text in label/registration certificate is given

bold italics: difference between label/registration certificate and government summary. Text in government overview is given

[1] 3-3.5 l/ha Agrol added

[2] formulation contains 40 g/kg diflubenzuron and 192 g/kg methomyl

[3] formulation contains 33 g/kg diflubenzuron and 180 g/kg methidathion

[4] dissolved in 5 l mineral oil

[5] 0.3% (v/v) mineral oil added

[6] 0.3% (v/v) crop oil added

[7] in combination with 50% (v/v) Agral

[8] 0.06% (v/v) summer oil added

[9] 0.3% (v/v) vegetable oil added

[10] 0.5 l/ha mineral oil (SOLEOL added)

[11] 0.5% (v/v) crop oil added

[12] 3 l/ha Renol added

Table 113. Registered pre-harvest uses of diflubenzuron on vegetables.

Crop	Site	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
				Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Broccoli	ns	Ireland	SC 240; SC 480	ns	**0.1**	**0.01-0.02**	2 **(21-28)**	14
Broccoli	ns	UK	WP 250; SC 480	ns	0.10	0.01-0.02	2	14
Brussels sprouts	ns	Ireland	SC 240; SC 480	ns	**0.1**	**0.01-0.02**	2 **(21-28)**	14
Brussels sprouts	ns	UK	SC 480; WP 250	ns	**0.1**	**0.01-0.02**	2	14
Cabbage	ns	Belarus *	WP 250	spray	0.12-0.25	ns	ns	30
Cabbage	ns	Chine	WP 250	spray	0.19-0.24	ns	ns	ns
Cabbage	ns	Cyprus	SC 480	ns	ns	0.01	ns	14
Cabbage	ns	Czech Republic	SC 480	spray	0.072	ns	ns	14
Cabbage	ns	Ireland	SC 240; SC 480	ns	**0.1**	**0.01-0.02**	2 **(21-28)**	14
Cabbage	ns	Jordan *	WP 250	ns	0.075-0.25	0.0075-0.025	ns	14
Cabbage	ns	Kazakhstan	SC 480	ns	0.029-0.048	ns	ns	ns
Cabbage	ns	Kenya	WP 250	**spray**	**0.1-0.125**	**0.01-0.012**	**ns**	**14**
Cabbage	ns	Lithuania	WP 250	**spray**	0.038	ns	ns	30
Cabbage	F	Poland	WP 250	**spray**	0.062	**0.01-0.016**	ns	14
Cabbage	ns	Portugal	WP 250	ns	ns	0.01	ns	14
Cabbage	ns	Romania *	WP 250	ns	0.12	0.0125-0.125	ns	14
Cabbage	ns	Russia	WP 250	ns	**0.038**	**0.0094-0.019**	1	30
Cabbage	ns	Slovakia	SC 480	ns	0.072	ns	ns	14

Crop	Site	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
				Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Cabbage	ns	Sweden	SC 480	ns	**0.072-0.096**	ns	ns	28
Cabbage	ns	Switzerland	SC 480	ns	**0.096**	0.01	ns	14
Cabbage	ns	Switzerland	WP 250	ns	ns	0.01	ns	14
Cabbage	ns	UK	SC 480; WP 250	ns	0.1	0.01-0.02	2	14
Calabrese	ns	Ireland	SC 240; SC 480	ns	**0.1**	**0.01-0.02**	2 **(21-28)**	14
Calabrese	ns	UK	WP 250; SC480	ns	0.1	0.01-0.02	2	14
Cauliflower	ns	Cyprus	SC 480	ns	ns	0.0096	ns	14
Cauliflower	ns	Ireland	SC 240; SC 480	ns	**0.1**	**0.01-0.02**	2 **(21-28)**	14
Cauliflower	ns	Jordan *	WP 250	ns	0.075-0.25	0.0075-0.025	ns	14
Cauliflower	ns	UK	WP 250; SC480	ns	0.1	0.01-0.02	2	14
Kale	ns	Kenya	WP 250	**spray**	**0.1-0.12**	**0.01-0.012**	ns	**14**
Mushrooms	I	Australia	WP 250	casing	5 g ai/bale	ns	ns	ns
Mushrooms	I	Australia	WP 250	compost	10 g ai /tonne	ns	ns	ns
Mushrooms	I	Australia	WP 250	**casing drench**	10	ns	ns	ns
Mushrooms	I	Belarus *	WP 250	spray	7.5	ns	ns	ns
Mushrooms	I	Belgium	SC 480; WP 250	HV **within 5 days of casing**	10	**0.05-0.1**	ns	ns
Mushrooms	I	Belgium	WP 250; SC 480	LV **within 5 days of casing**	10	1.0	ns	ns
Mushrooms	I	Cyprus	SC 480	ns	9.6	ns	ns	14
Mushrooms	I	Czech Republic	SC 480	spray	7.2-9.6	ns	ns	14
Mushrooms	I	Denmark	WP 250	spray	10	**0.025-0.1**	ns	ns
Mushrooms	I	France	SC 150	compost or casing	**10**	**0.2-1.0**	ns	ns
Mushrooms	I	Germany @	WG 800	compost and casing	5.6	0.037-0.056	2	ns
Mushrooms	I	Greece	WP 250	ns	10	0.04	ns	ns
Mushrooms	I	Hungary	WP 250	**compost**	**7.5-10**	**0.75-1.0**	ns	21
Mushrooms	I	Ireland	SC 240	**at casing**	9.6	**0.038**[1]	1	[2]
Mushrooms	I	Ireland	WP 250	**at casing**	10	ns	ns	ns
Mushrooms	I	Italy	WP 050	**at casing**	**10**	**0.04**	ns	ns
Mushrooms	I	Jordan *	WP 250	ns	ns	0.038-0.05	ns	14
Mushrooms	I	Kenya	WP 250	**at casing**	**10**	**0.04**	ns	7
Mushrooms	I	Kenya	WP 250	**compost**	**10 g ai/cu. m**^{3}	**ns**	ns	7
Mushrooms	I	Netherlands @	SC 480; WP 250	**compost or casing**	***9.6*-10**	**0.06-1.0**	**1**	ns
Mushrooms	I	Norway	SC 480	**at casing**	**9.6**	0.038	ns	[2]
Mushrooms	I	Poland	WP 250	**within 3 days after casing**	8.8-10	**0.058-0.10**	ns	ns
Mushrooms	I	Romania *	WP 250	ns	10	ns	ns	ns
Mushrooms	I	Russia	WP 250	**spray**	7.5	ns	2	25
Mushrooms	I	Saudi Arabia *	WP 250	ns	10	ns	ns	ns
Mushrooms	I	Slovakia	SC 480	**spray**	7.2-9.6	ns	ns	14
Mushrooms	I	South Africa	WP 250	after casing	10	0.1	ns	ns
Mushrooms	I	Spain	WP 250	**after casing**	10	**0.033**	ns	ns
Mushrooms	I	Sweden	WP 250	ns	10	ns	ns	21
Mushrooms	I	Switzerland	SC 480	**at casing**	9.6	**0.048**	ns	ns
Mushrooms	I	Switzerland	WP 250	ns	10	ns	ns	ns
Mushrooms	I	UK	SC 480; WP 250	**at casing**	10	**0.04**[1]	1	[2]

Crop	Site	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
				Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Mushrooms	I	United Arab Emirates	WP 250	ns	1	ns	ns	3
Mushrooms	I	USA	SC 480; WP250	compost and/or casing	29-49 10	ns 0.063	ns	ns
Pepper	G	Poland	WP 250	**spray**	**0.1-0.2**	0.01	ns	7
Tomato	ns	Brazil *	WP 250	spray	0.125	ns	1-2	4
Tomato	ns	Chile	SC 480	spray; before fruit setting	0.036-0.06	ns	1-2	ns
Tomato	ns	Ecuador	WP 250	spray	0.0625-0.12	ns	ns	14
Tomato	ns	Greece	WP 250	ns	ns	0.01-0.025	ns	45
Tomato	G	Poland	WP 250	**spray**	**0.1-0.2**	0.01	ns	7
Tomato	ns	Uruguay	WP 250; SC 480	spray	0.12	ns	ns	15
Vegetables	ns	Greece	WP 250	ns	ns	0.01-0.025	ns	45
Vegetables	ns	Guatemala	WP 250	ns	0.1	0.01-0.02	ns	8
Vegetables	ns	Ireland	WP 250	ns	0.1	**0.01-0.02**	**1-2**	ns
Vegetables	ns	Saudi Arabia *	WP 250	ns	ns	0.0025-0.02	ns	ns
Vegetables	ns	Syria *	WP 250	ns	ns	0.01-0.02	ns	ns
Vegetables	ns	United Arab Emirates	WP 250	ns	ns	0.01-0.02	ns	3

ns: not specified
F: field
G: glasshouse
I: indoor
* no printed label or registration certificates available (confirmed by applicant)
@ information on GAP provided by the national government in summarized form
bold: difference between label/registration certificate and applicant's summary. Text in label/registration certificate is given
[1] quantities are based on a casing weight of 1 tonne/30.5 m^2 and will provide a dose of 30 mg ai/kg
[2] latest application immediately after casing

Table 114. Registered pre-harvest uses of diflubenzuron on pulses, cereal grains, oilseed and forage or fodder crops grown in the field.

Crop	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
			Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Alfalfa/ lucerne	Saudi Arabia *	WP 250	ns	ns	0.0025-0.02	ns	14
Barley	Hungary	WP 250	spray	0.075	**0.025**	ns	ns
Barley	Hungary	WP 250	aerial spray	0.075	**0.094-0.15**	ns	ns
Barley	Poland	WP 250	**spray**	0.075	**0.02-0.05**	ns	10
Cereals	Switzerland	SC 480	ns	0.12	ns	ns	42
Barley	Uruguay	WP 250; SC 480	spray	0.025	ns	ns	ns
Clover	Greece	WP 250	ns	ns	0.01-0.025	ns	21
Cotton	Argentina	WP 250	aerial spray	0.05	0.12-0.2	1-2	45
Cotton	Brazil	WP 250	spray	**0.075-0.015**	ns	1-2	28
Cotton	Columbia	WP 250	aerial spray	0.038-0.075	ns	4-6	ns
Cotton	Ecuador	WP 250	spray	0.025-0.1	ns	ns	ns
Cotton	Greece	WP 250	ns	ns	0.01-0.025	ns	21
Cotton	Guatemala	WP250	ns	0.075	ns	ns	ns
Cotton	Kazakhstan	SC 480	ns	0.048-0.48	ns	ns	ns
Cotton	Pakistan	WP 250	ns	0.19-0.25	ns	3-4 (15-20)	ns
Cotton	Peru	WP 250	ns	0.075-0.012	0.019-0.031	ns	3-5
Cotton	Saudi Arabia *	WP 250	ns	ns	0.0025-0.005	ns	ns
Cotton	Syria *	WP 250	ns	0.075	ns	ns	ns

Crop	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
			Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Cotton	USA	OF 240; SC 240; WP 250	LV spray	0.035-0.14 (total max 0.42/season)	0.019-0.15	6 (5-14)	14
Cotton	USA	OF 240; SC 240	ULV spray	0.035-0.14 (total max 0.42/season)	0.75-9.6	6 (5-14)	14
Cotton	USA	OF 240; SC 240; WP250	LV aerial spray	0.035-0.14 (total max 0.42/season)	0.075-0.50	6 (5-14)	14
Cotton	USA	OF 240; SC 240	ULV aerial spray	0.035-0.14 (total max 0.42/season)	1.0-9.6	6 (5-14)	14
Field crops	Greece	WP 250	ns	ns	0.01-0.025	ns	21
Field crops	United Arab Emirates	WP 250	ns	ns	0.01-0.02	ns	3
Maize/corn	Brazil *	WP 250	spray	0.025	ns	1-2	60
Maize/corn	Ecuador	WP 250	spray	0.062-0.075	ns	ns	14
Maize/corn	France	SC 150	ns	0.06-0.12	ns	1-2 (10)	ns
Maize/corn	France	WP 250	ns	0.12	ns	ns	ns
Maize/corn	Peru	WP 250	ns	0.075	0.019	ns	3-5
Maize/corn	Uruguay	SC 480	spray	0.024-0.029	ns	ns	ns
Maize/corn	Uruguay	WP 250	spray	0.025-0.03	ns	ns	ns
Oats	Poland	WP 250	**spray**	0.075	**0.019-0.05**	ns	10
Pasture/ Rangeland	Chile	SC 480	spray	0.036-0.06	0.018-0.03	1	ns
Pasture/ Rangeland	Kazakhstan	OF 060	**ULV spray**	**0.006**	ns	ns	ns
Pasture/ Rangeland	Russia	WP 250	ns	0.012-0.035	ns	1	20
Pasture/ Rangeland	Spain	SU 009; SU 015	ULV aerial spray	0.045	ns	ns	3-7
Pasture/ Rangeland	USA	OF 240; SC 240	LV spray	0.0088-0.018 (total max 0.018)	0.0047-0.038[1]	1-2 (14-21)	ns
Pasture/ Rangeland	USA	OF 240; SC 240; WP 250	LV aerial spray	0.0088-0.018 (total max 0.018)	0.019-0.19[1]	1-2 (14-21)	ns
Pasture/ Rangeland	USA	OF 240; SC 240	ULV aerial spray	0.0088-0.018 (total max 0.018)	0.38-2.0[1]	1-2 (14-21)	ns
Pasture/ Rangeland	Uzbekistan *	OF 060; SC 480	ns	0.03-0.06	ns	ns	ns
Potato	Greece	WP 250	ns	ns	0.01-0.025	ns	45?
Rice	Columbia	WP 250	spray	0.062	ns	1	ns
Rice	Ecuador	WP 250	spray	0.062-0.075	ns	ns	14
Rice	USA	SC 240	LV aerial spray	0.14-0.28	0.30-0.60	1-2 (5-7)	80 (14 water)
Rye	Poland	WP 250	spray	0.075	0.019-0.050	ns	10
Soya bean	Brazil	WP 250	spray	0.015-0.02	ns	1	21
Soya bean	Columbia	WP 250	spray	0.025	ns	1	ns
Soya bean	Ecuador	WP 250	spray	0.02-0.1	ns	ns	14
Soya bean	Guatemala	WP 250	ns	0.038-0.075	ns	ns	8
Soya bean	Saudi Arabia *	WP 250	ns	ns	0.0025-0.005	ns	ns
Soya bean	Uruguay	WP 250; SC 480	spray	0.02-0.03	ns	ns	21
Soya bean	USA	OF 240; SC 240	LV spray	0.035-0.070	0.011-0.083[2]	1-2 (30)	21
Soya bean	USA	OF 240; SC 240	LV aerial spray	0.035-0.070	0.075-0.25[2]	1-2 (30)	21
Sorghum	Columbia	WP 250	spray	0.062	ns	1	ns
Sugar beet	Macedonia	WP 250	ns	0.20-0.25	**0.033-0.083**	ns	35

Crop	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
			Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Sugar beet	Yugoslavia *	WP 250	ns	0.20-0.25	ns	2 (14-21)	35
Triticale	Poland	WP 250	**spray**	0.075	**0.019-0.050**	ns	10
Wheat	Bulgaria	WP 250	ns	0.12	ns	ns	30
Wheat	Chine	WP 250	spray	0.022-0.075	ns	ns	ns
Wheat	Hungary	WP 250	spray	0.075	**0.025**	ns	ns
Wheat	Hungary	WP 250	aerial spray	0.075	**0.094-0.15**	ns	ns
Wheat	Poland	WP 250	**spray**	0.075	**0.019-0.050**	ns	10

ns: not specified
* no printed label or registration certificates available (confirmed by applicant)
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bold: difference between label/registration certificate and applicant's summary. Text in label/registration certificate is given

[1] at high air temperature or at low humidity use 1.2-4.7 l/ha cotton seed oil, vegetable oil or paraffinic crop oil for LV spray or LV aerial spray and dissolve in 0.88-2.3 l/ha oil carrier for ULV aerial spray.

[2] at high air temperature or at low humidity use 1.2-9.4 l/ha cotton seed oil, vegetable oil or paraffinic crop oil for LV spray or LV aerial spray and use 0.6-1.5 l/ha cottonseed oil or vegetable or petroleum based carriers for ULV spray.

Table 115. Registered pre-harvest uses of diflubenzuron on forests (wild berries/wild mushrooms/herbs). All field grown.

Crop	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
			Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Forest	Albania *	SC 480	ns	0.036-0.072	ns	ns	ns
Forest	Algeria	WP 250	**aerial spray**	**0.15**	**0.6**	ns	ns
Forest	Algeria *	OF 450	ns	0.05	1.0[1]	ns	ns
Forest	Austria	WP 250	**spray**	0.038-0.075	**0.0062-0.075**	**1-2**	ns
Forest	Austria	WP 250	**aerial spray**	0.038-0.075	**0.075-0.25**	**1-2**	ns
Forest	Belarus *	WP 250	spray	0.025-0.25	ns	ns	ns
Forest	Belgium	WP 250; SC 480	ns	ns	0.003-0.03	ns	ns
Forest	Belgium *	OF 450	ULV spray	0.072	1.4-3.6[2]	ns	ns
Forest	Bulgaria	SC 480	ULV spray	0.048	1.6[3,4,5]	ns	30
Forest	Chine	WP 250	spray	ns	0.004-0.006	ns	ns
Forest	Chine	WP 250	ULV spray	0.030-0.045	ns	ns	ns
Forest	Cyprus	WP 250	aerial spray	**0.062-0.075**	ns	ns	30
Forest	Cyprus	WP 250	**spray**	**ns**	**0.0075**	ns	30
Forest	Cyprus	SC 480	spray	0.072	ns	ns	30
Forest	Czech Republic	SC 480	ULV spray	**0.058-0.086[6]**	ns	ns	**21**
Forest	Estonia	SC 480	ULV spray	0.072	2.5-3.4[7]	ns	ns
Forest	Estonia	WP 250	ULV spray	0.025-0.075	0.93-3.8[7]	ns	ns
Forest	Finland	SC 480	aerial spray	0.072	**0.14**	ns	1 year[12]
Forest	Finland	SC 480	spray	0.072	**0.014-0.072**	ns	1 year[12]
Forest	Finland *	WP 250	aerial spray	0.075	0.15	ns	ns
Forest	Finland *	WP 250	spray	0.075	0.05-0.08	ns	ns
Forest	France	OF 450	aerial spray	0.056-0.068	ns[8]	ns	ns
Forest	France	OF 450	**ULV aerial spray**	0.038	**ns[8]**	ns	ns
Forest	France	OF 450; WP 250	spray	0.074	ns[8]	ns	ns
Forest	France	SC 480	**ULV aerial spray**	0.048	1.6[9]	ns	ns
Forest	France *	OF 450; WP 250	fogging	0.075	ns	ns	ns
Forest	Germany @	WG 800	spray	0.06	0.01-0.06	1	ns
Forest	Germany @	WG 800	aerial spray	0.06	**0.12-0.20**	1	ns
Forest	Greece	WP 250	spray	ns	**0.0075-0.015**	ns	ns

Crop	Country	Form (g ai/l or g ai/kg)	Application				PHI, days
			Method	Rate kg ai/ha	Spray conc, kg ai/hl	Interval, days	
Forest	Greece *	OF 450	ns	ns	1.12-3.38[10]	ns	ns
Forest	Guatemala	WP 250	ns	0.075	ns	ns	ns
Forest	Hungary	OF 450	**ULV spray; ULV aerial spray**	0.05-0.074	**0.99-3.7[14]**	ns	ns
Forest	Hungary	WP 250	ns	0.050-0.125	**0.0033-0.012**	ns	ns
Forest	Iran *	WP 250	ns	0.10	ns	ns	ns
Forest	Ireland	SC 240; SC 480	**spray**	0.072	ns	1	ns
Forest	Ireland	SC 480	**ULV aerial spray**	0.072	**0.72-7.2[11]**	1	ns
Forest	Ireland	WP 250	ns	0.038-0.075	ns	ns	ns
Forest	Italy	WP 050; WP 250	ns	ns	0.012-0.018	ns	ns
Forest	Jordan	WP 250	ns	ns	0.0038-0.005	ns	ns
Forest	Kazakhstan	SC 480	ns	0.043-0.058	ns	ns	ns
Forest	Lithuania	SC 480	**spray**	0.1-0.14	ns	ns	4[12] (1 re-entry)
Forest	Lithuania	WP 250	**spray**	0.025-0.075	ns	ns	4[12] (1 re-entry)
Forest	Macedonia	WP 250	ns	0.075	ns	ns	ns
Forest	Moldova	WP 250	ns	0.01	ns	ns	ns
Forest	Morocco	SC 480	**ULV spray**	**0.048**	**1.6[13]**	**ns**	ns
Forest	Morocco *	OF 450	ULV spray	0.045	0.90[8]	ns	ns
Forest	Morocco *	WP 250	ns	0.15-0.20	ns	ns	ns
Forest	Pakistan	WP 250	ns	ns	0.01-0.02	ns	ns
Forest	UK	SC 480	**ULV spray**	0.072	**0.72-7.2**	1	ns
Forest	UK	WP 250	ns	0.075	ns	1	ns

ns: not specified
* no printed label or registration certificates available (confirmed by applicant)
@ information on GAP provided by the national government in summarized form
bold: difference between label/registration certificate and applicant's summary. Text in label/registration certificate is given
[1] dissolved in 5 l mineral oil
[2] dissolved in 2-5 l of oil
[3] 8.3% (v/v) mineral oil EC (Pavolin) added
[4] 16.7% (v/v) Akarzin added
[5] 16.7 % (v/v) Pavolin (mineral oil) added
[6] 6.6 l/ha mineral oil and 3.3 l/ha Dedal 90 EC or 3.3 l/ha Ikar 95EC or 3.3 l/ha Istroekol added
[7] 0.3% (v/v) mineral oil added
[8] 2-5 l/ha diesel oil or crop oil added
[9] 0.7 l/ha Banole Total (crop oil) added
[10] dissolved in 200-500 ml of diesel oil/stremma
[11] add Actipron or other suitable adjuvant
[12] pre harvest interval for wild berries, wild mushrooms and medicinal herbs
[13] 1 l/ha Citrole 60 added (=33% (v/v) oil)
[14] dissolved in FK15 paraffin oil or gas oil (2-5 l/ha)

Table 116. Registered uses of diflubenzuron on livestock and fish.

Livestock/fish	Country	Trade name	Application				Withdrawal period (days)
			Method	Rate	Rate, mg ai/kg bw/day	No.	
Atlantic salmon (Salmo salar)	Norway *	Lepsidon 0.6 g ai/kg	medicated pellets	-	0.018	2-3/year; during 14 days	60

Livestock/fish	Country	Trade name	Application				Withdrawal period (days)
			Method	Rate	Rate, mg ai/kg bw/day	No.	
Cattle	Australia@	Stampede, pour on; 20 g ai/l	body strike[6]	10-40 ml of product per 100 kg bw	0.2-0.8 g ai/100 kg bodyweight		21 (slaughter), 0 (milk)
Goats	South Africa	Fleececare 250 g ai/l	plunge dip[2] with mechanical mixing	0.625 g ai/l; 400 L for 20 beasts	-	2/day[3]	none[1]
Goats	South Africa	Fleececare 250 g ai/l	hand jetting; skin saturation	0.625 g ai/l; 2-5 l/beast	-	ns[3]	none[1]
Pigs	France *	Duphacid; 100 g ai/kg	feed-through	0.75 mg ai/kg feed	-	continuous	ns
Pigs	France *	Kitinex; 100 g ai/kg	feed-through	0.75 mg ai/kg feed	-	continuous	ns
Poultry	France *	Duphacid; 100 g ai/kg	feed-through	1.5 mg ai/kg feed	-	continuous	ns
Poultry	France *	Kitinex; 100 g ai/kg	feed-through	1.5 mg ai/kg feed	-	continuous	ns
Sheep	Australia@	Backlash pour-on, 20 g ai/l	ns	12-30 ml of product per sheep	0.24-0.6 g ai/animal		21 (slaughter), milk not for consumption
Sheep	Australia@	Magnum, pour-on, 25 g ai/l	body or body & crutch[4]	12-51 ml of product per sheep	0.3-1.28 g ai/animal		42 (slaughter), milk not for consumption
Sheep	Australia@	Strike, 250 g ai/l	plunge dip, shower dip or hand jetting[5]	150-200 ml product /hl	0.038-0.05 kg ai/hl		None (slaughter), none (milk)
Sheep	Australia@	Fleececare; IGR jet and dip; Duodip; 250 g ai/l	dip or spray	150-200 ml product /hl	0.038-0.05 kg ai/hl		None (slaughter), none (milk)
Sheep	Australia@	Crusader; conc ns	ns	40 ml product /hl	ns		ns
Sheep	South Africa	Fleececare 250 g ai/l	plunge dip[2] with mechanical mixing	0.625 g ai/l; 400 L for 20 beasts	-	2/day[3]	none[1]
Sheep	South Africa	Fleececare 250 g ai/l	hand jetting; fleece saturation	0.625 g ai/l; 2-5 l/beast	-	ns[3]	none[1]

* no printed label available

@ information on GAP provided by national government in summarized form.

[1] Ewes should be treated not later than 6 weeks before lambing/kidding as live lice can infect their lambs/kids.

[2] For control of red lice dip 10-14 days after shearing. For treatment of red lice dip when the problem becomes obvious (14 days – 10 months after shearing). For the control of blowfly dip on or before the onset of a fly wave. Zinc sulfate may be added at a rate of 2.5 g/l. Ewes heavy in lamb may not be dipped.

[3] Sheep and goats will be protected from fly-strike and lice build-up for up to 16 weeks. With heavy soiling of the crutch it may be necessary to retreat at 10 weeks intervals.

[4] Product must not be used on lactating or pregnant ewes where milk or milk products may be used for human consumption. For treatment of sheep lice, product must be used within 24 hours after shearing or on lambs not older than 3 months. Dose ml product per animal for treatment of sheep lice: 8 (<10 kg), 10 ml (11-20 kg), 15 ml (21-30 kg), 20 ml (31-55 kg), 25 ml (56-75 kg), add 5 ml for each 10 kg above 75 kg.

For treatment of blowfly, product is only for use on sheep with 6 weeks – 6 months wool growth. Product must not be used less than 6 months before shearing or fibre collection. Dose 2 x 17 ml bands on either side of the backline (34 ml/sheep) or additionally dose 1 x 17 ml from the crutch to the top of the tail (51 ml/sheep).

[5] Product must not be used within 10 days after shearing. Use dipping for the period 10-42 days after shearing, use hand jetting for the period 43 days – 6 months after shearing. Ensure saturation of the skin. Plunge dips should be at least 10 m in length, mechanical mixing should be carried out and sheep have to be pushed under twice. Shower dips will require at least

10 min for sheep with fine and long wool. For hand jetting apply 0.5 L diluted jetting fluid per month of wool growth per sheep (minimum 2.5 l/sheep). Zinc sulfate may be added for the control of lumpy wool. Product must not be used less than 6 months before shearing or fibre collection.

[6] Apply along the midline of the back between the wither and the base of the tail. Dose rate of product: 25 ml (<100 kg bw), 40 ml (101-200 kg bw), 50 ml (201-300 kg bw), 60 ml (301-400 kg), 70 ml (401-500 kg). Add an additional 10 ml for each 100 kg bw above 500 kg.

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

The Meeting received information on diflubenzuron supervised field trials on

Fruits	Table 117	Grapefruit, lemon, lime, mandarin, orange
	Table 118	Apple
	Table 119	Pear
	Table 120	Peaches
	Table 121	Plums
	Table 122	Blackcurrants, gooseberries
Vegetables	Table 123	Brussels sprouts, head cabbage
	Table 124	Sweet peppers, Chilli peppers, tomatoes
	Table 125	Mushrooms
Pulses	Table 126	Peas, soya bean
Cereal grains	Table 127	Rice
Tree nuts	Table 128	Walnuts, almonds, pecans
Oil seeds	Table 129	Cotton
Forage crops	Table 130	Range grass

Application rates were reported as diflubenzuron. Residue levels were reported as mg/kg of the compound determined (parent, CPU, PCA or DFBA). Values below the reported LOQ are indicated as e.g. <0.05 when the LOQ is reported as 0.05 mg/kg. This is not necessarily the validated LOQ. Residues, application rates and spray concentrations have been rounded to two figures.

Results from replicate field plots are presented as individual values. Results from replicate field samples are presented as individual values followed by the mean. Results from replicate laboratory samples and replicate analytical portions are presented as the means. Residue values from the trials conducted according to maximum GAP have been used for the estimation of maximum residue levels and STMRs. These levels are double-underlined. Where GAP, but not maximum GAP, was used to derive an STMR the residues are single-underlined.

The trials included control plots and analytical recovery checks with spiked control samples. The results of the analyses are recorded in the Residue Analysis section (Tables 48-93). Residues in the tables are presented as uncorrected for matrix interferences or recoveries, where uncorrected values were available.

For the GLP trials, laboratory reports included method validations with batch recoveries at residue levels similar to those occurring in samples from the supervised trials. Dates of analyses or duration of sample storage were also provided. Field reports provided data on the sprayers used and their calibration, plot sizes, residue sample sizes and sampling dates.

Citrus fruits (group 001)

Results of trials on citrus fruits are shown in Table 117. US trials in 1975-1976 (non-GLP) on oranges, grapefruit and tangerines were not included as the data were presented in summary form and original reports were not available. The results of trials in the USA in 1978 (Nigg *et al.*, 1986, non-GLP) on oranges were not included because the results were reported as µg/cm^2.

In trials on oranges in the USA in Clovis and Fresno in 1985 (Duphar, 1986a,b, non-GLP) the samples were analysed for diflubenzuron using GC-ECD method LAI 3-86-6. Residues were corrected for concurrent method recoveries in Fresno (77%-102%), but not in Clovis (119%) and not for matrix interferences (<0.05 mg/kg LOQ) at either site (Table 74). In trials in the USA in 1988/1989 (Van Zijtveld *et al.*, 1989, non-GLP) on oranges and grapefruit the samples were analysed by GC-ECD method LAI 3-86-6 for diflubenzuron and method RES027 for PCA; CPU was not determined. Residues were not corrected for concurrent method recoveries (85%-86% for diflubenzuron, 13%-46% for PCA) or matrix interferences (max 0.020 mg/kg diflubenzuron and 0.00032 mg/kg PCA in oranges) (Tables 74 and 86). Because of low storage stabilities (37%-71% for diflubenzuron at 0.2-1.0 mg/kg and 0%-35% for PCA at 0.01 mg/kg) the results could not be evaluated.

In trials in the USA in 1996 (Gaydosh 1999a, GLP) on lemons and limes samples were analysed for diflubenzuron by GC-ECD (Duphar, 1990). Residues were not corrected for concurrent method recoveries (86%-112%) or matrix interferences (max. 0.046 mg/kg diflubenzuron in limes) (Table 78). Because of matrix interferences results below 0.2 mg/kg are not considered valid.

In trials in Spain in 1995 (Van Dijk and Keetelaar-Janssen, 1995; Van Dijk, 1996, non-GLP) on mandarins and oranges the samples were analysed for diflubenzuron using HPLC method RES029. Residues were corrected for concurrent method recoveries (81%-91%), but not for matrix interferences (<0.01 mg/kg = LOQ) (Table 60).

In trials in Italy in 1996 and 1997 (Allan *et al.*, 1997, non-GLP; Allan and Thus, 1999, non-GLP) on lemons and oranges the reports on field conditions, weather conditions and sampling were not available to the reviewer (only the intended field protocols were available). The samples were analysed for diflubenzuron by HPLC method RES045. Residues were not corrected for concurrent method recoveries (85%-106% in 1996, 46%-86% in 1997) or matrix interferences (<0.02 mg/kg = LOQ in 1986, max 0.027 mg/kg in 1997, Table 61). Because of low concurrent method recoveries the 1997 results could not be evaluated.

Table 117. Residues of diflubenzuron and PCA in unwashed whole citrus fruit from applications close to harvest; double underlined results are according to critical GAP for that country or a neighbouring country.

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	interval, days	PHI, days	diflubenzuron, mg/kg	PCA, mg/kg	Ref
Grapefruit								
USA, Ft Pierce (FL), 1988/89, white grapefruit	WP 250	1x 0.35	?	-	21	0.093	<0.001	3
USA, Avon park (FL), 1988/89, Duncan	WP 250	1x 0.35	?	-	21	0.27	<0.001	3
USA, Avon park (FL), 1988/89, Marsh Seedless	WP 250	1x 0.35	?	-	21	0.20	<0.001	3
USA, Ft Pierce (FL), 1988/89, Red Grapefruit	WP 250	3x 0.35	3x0.0062	69, 160	21	0.16	<0.001	3-
USA, Ft Pierce (FL), 1988/89, Red Marsh	WP 250	3x 0.35	1x0.0061 2x0.0075	78, 133	21	0.13	<0.001	3
USA, Winter Haven (FL), 1988/89, Grapefruit	WP 250	3x 0.35	1x0.0075 2x0.015	41, 149	21	0.069	<0.001	3
USA, Donna (TX), 1988/89, Henderson Ruby Red	WP 250	3x 0.35	3x0.015	61, 85	85[1] 21	<0.05 0.27	<0.001 <0.001	3
USA, South Res. Farm (TX), 1988/89, Red blush	WP 250	3x 0.35	3x0.015	60, 198	21	0.16	<0.001	3

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	interval, days	PHI, days	diflubenzuron, mg/kg	PCA, mg/kg	Ref
USA, Innokalee (FL), 1988/89, White grapefruit	WP 250	3x 0.35	3x0.053	74, 160	21	0.19	<0.001	3
USA, Innokalee (FL), 1988/89, Red grapefruit	WP 250	3x 0.35	3x0.053	75, 157	21	0.27	<0.001	3
Lemon								
USA, Fallbrook (CA), 1996, Eureka	SC 480	3x 0.35	3x0.030	89, 91	21	0.24 (2); mean 0.24	na	4
USA, Fallbrook (CA), 1996, Eureka	WP 250	3x 0.35	3x0.030	89, 91	21	0.54, 0.55; mean 0.55	na	4
Italy, Sicily, 1996, -	WP 040 + MTM	2x 0.24	2x0.012	30	30[2] 0 14 28	0.27, 0.30; mean 0.28 0.46, 0.68; mean 0.57 0.28, 0.29; mean 0.28 0.21, 0.26; mean 0.24	na	5
Italy, Sicily, 1997, -	WP 040 + MTM	2x 0.24	2x0.012	30	30[2] 0 14 28	0.10 0.34 0.21 0.17	na	6
Italy, Sicily, 1996, -	WP 050	2x 0.25	2x0.012	30	30[2] 0 14 28	0.11, 0.18; mean 0.14 0.20, 0.42; mean 0.31 0.22, 0.27; mean 0.24 0.16, 0.19; mean 0.18	na	5
Italy, Sicily, 1997, -	WP 050	2x 0.25	2x0.012	30	30[2] 0 14 28	0.084 0.33 0.16 0.15	na	6
Italy, Sicily, 1996, -	WP 250	2x 0.38	2x0.019	30	30[2] 0 14 28	0.26, 0.28; mean 0.27 0.42, 0.49; mean 0.46 0.28, 0.39; mean 0.34 0.24, 0.29; mean 0.26	na	5
Italy, Sicily, 1997, -	WP 250	2x 0.38	2x0.019	30	30[2] 0 14 28	0.14 0.55 0.42 0.35	na	6
Lime								
USA, Immokalee (FL), 1996, Eureka	SC 480	3x 0.35	3x0.030	90, 90	21	0.16, 0.17; mean 0.17	na	4
US, Immokalee (FL, 1996, Eureka	WP 250	3x 0.35	3x0.030	90, 90	21	0.15, 0.16; mean 0.16	na	4
Mandarin								
Spain, Quart de Poblet, 1995, Fortuna	WP 250	1x 0.36	1x0.015	-	0 7 14 21 28	0.30 0.43 0.28 0.25 0.33	na	7
Spain, Moncada, 1995, Fortuna	WP 250	1x 0.38	1x0.015	-	0 7 14 21 28	0.27 0.29 0.27 0.25 0.18	na	7
Orange								
USA, Clovis (CA), 1985, -	WP 250	3x 0.35	3x0.019	32, 71	21	0.13, 0.14, 0.16, 0.18 (2)	na	8
USA, Clovis (CA), 1985, -	WP 250	3x 1.4	3x0.075	32, 71	21	0.20, 0.26 (3), 0.27	na	8
USA, Fresno (CA) 1985, Valencia	WP 250	1x 0.35	1x0.0094	-	7 14 21	<0.05, 0.15 (2), 0.20, 0.23, 0.35 0.06, 0.07, 0.10 (2), 0.19, 0.23 <0.05, 0.08, 0.12 (2), 0.14, 0.22	na	9

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	interval, days	PHI, days	diflubenzuron, mg/kg	PCA, mg/kg	Ref
USA, Fresno (CA) 1985, Valencia	WP 250	1x 1.4	1x0.037	-	7 14 21	0.15 (2), 0.23, 0.31, 0.43, 0.77 0.07, 0.10 (2), 0.37, 0.59, 0.90 0.08, 0.12, 0.14, 0.65, 0.72, 0.89	na	9
USA, Avon park (FL), 1988/89, Valencia	WP 250	1x 0.35?	?	-	21	0.24	<0.001	3
USA, Avon park (FL), 1988/89, Pineapple	WP 250	1x 0.35?	?	-	21	0.30	<0.001	3
USA, Avon park (FL), 1988/89, Valencia	WP 250	1x 0.35	?	-	21	0.16	<0.001	3
USA, Ft Pierce (FL), 1988/89, Valencia	WP 250	3x 0.35	3x0.0062	68, 156	21	0.27	<0.001	3
USA, Ft Pierce (FL), 1988/89, Pineapple	WP 250	3x 0.35	3x0.0062	68, 156	21	0.26	<0.001	3
USA, Ft Pierce (FL), 1988/89, Valencia	WP 250	3x 0.35	3x0.0075	83, 135	21	0.14	<0.001	3
USA, Winter Garden (FL), 1988/89, Orlando tangelo	WP 250	3x 0.35	3x0.0075	71, 139	21	0.16	<0.001	3
USA, Winter Garden (FL), 1988/89, Temple	WP 250	3x 0.35	3x0.0075	71, 139	21	0.22	<0.001	3
USA, Sanford (FL), 1988/89, Hamlin	WP 250	3x 0.35	3x0.0075	77, 118	21	0.24	<0.001	3
USA, Winter Haven (FL), 1988/89, Hamlin	WP 250	3x 0.35	1x0.0075 2x0.015	41, 149	21	0.16	<0.001	3
USA, Innokalee (FL), 1988/89, Hamlin	WP 250	3x 0.35	3x0.053	55, 160	21	0.11	<0.001	3
Spain, Quart de Poblet, 1995, Navel	WP 250	1x 0.45	1x 0.015	-	0 7 14 21 28	0.34 0.42 0.32 0.34 0.28	na	7
Spain, Moncada, 1995, Valencia	WP 250	1x 0.46	1x0.015	-	0 7 14 21 28	0.38 0.28 0.36 0.32 0.27	na	7
Italy, Sicily, 1996, -	WP 040 + MTM	2x 0.24	2x0.012	30	30[2] 0 14 28	0.12, 0.15; mean 0.14 0.31, 0.35; mean 0.33 0.22, 0.27; mean 0.24 0.18 (2); mean 0.18	na	5
Italy, Sicily, 1997, -	WP 040 + MTM	2x 0.24	2x0.012	30	30[2] 0 14 28	0.12 0.46 0.23 0.19	na	6
Italy, Sicily, 1996, -	WP 050	2x 0.25	2x0.012	30	30[2] 0 14 28	0.24, 0.29; mean 0.26 0.41 (2); mean 0.41 0.31, 0.40; mean 0.36 0.24, 0.30; mean 0.27	na	5
Italy, Sicily, 1997, -	WP 050	2x 0.25	2x0.012	30	30[2] 0 14 28	0.18 0.44 0.24 0.19	na	6
Italy, Sicily, 1996, -	WP 250	2x 0.38	2x0.018	30	30[2] 0 14 28	0.29, 0.83; mean 0.56 0.59, 1.1; mean 0.85 0.50, 0.52; mean 0.51 0.44, 0.46; mean 0.45	na	5
Italy, Sicily, 1997, -	WP 250	2x 0.38	2x0.018	30	30[2] 0 14 28	0.22 0.88 0.42 0.28	na	6

[1] After 2nd treatment, just before 3rd

[2] Just before 2nd treatment

[3] DI-7492, Van Zijtveld *et al.*, 1989, non-GLP; spray volume 655-5705 l/ha using a handgun; weather conditions and soil not stated; harvest Jan/Feb 89; mature fruits; 25 fruits/sample; plot size not stated. Storage at –20°C for 133 days (diflubenzuron) or 2 days (PCA). Fruits from Donna (TX) were also harvested just before the third treatment (85 days after second).

[4] RP-96028, Gaydosh, 1999a, GLP; spray volume 1168 l/ha; tractor-mounted equipment; no unusual weather conditions (Jun-Jan in Immokalee and Nov-Jun in Fallbrook, temp -2-35°C; total rain 23-62 cm); soil not stated; harvest Jan 97 (Immokalee) or Jun 97 (Fallbrook); fruit selection from 6 designated locations within each tree, 4-6 trees per plot; plot size 220-350 m^2; 2 field samples/plot; 24 fruits, 2.7-4.5 kg/sample, storage frozen for 13-161 days (temperature not stated).

[5] DI-10942, Allan *et al.*, 1997, GLP not confirmed because a field report was not available (only a field protocol); decline trial; in one of the applications 1.152 kg ai/ha methomyl was included; spray volume 2000 l/ha; overall foliar spray to run-off; weather conditions, soil and plot size not stated; harvest June/July 96; 2 field samples per plot; 24 fruits/sample (>2 kg) from at least 4 trees. Storage 7-9 months at –10°C.

[6] DI-11424, Allan and Thus., 1999, GLP not confirmed because a field report was not available (only a field protocol); decline trial; in one of the applications 1.152 kg ai/ha methomyl was included; spray volume 2000 l/ha; overall foliar spray to run-off; weather conditions and soil not stated; harvest June/July 97; plot size not stated; 24 fruits/sample (>2 kg) from at least 4 trees. Storage 6-8 months at –10°C.

[7] DI-9331, Van Dijk and Keetelaar-Janssen, 1995, non-GLP and Van Dijk, 1996, non-GLP; decline trial; spray volume 2400-3100 l/ha with the addition of 0.5% emulsive oil; gun spray; weather conditions and soil not stated; harvest Jan/Feb 95; plot and sample size not stated. Storage 22-57 days at –18°C.

[8] DI-5295, Duphar, 1986a, non-GLP; spray volume 1869 l/ha; equipment, weather conditions and soil type not stated; harvest Dec 85, samples from 5 replicate field plots; storage 30 days (temperature, plot and sample size not stated).

[9] DI-5514, Duphar, 1986b, non-GLP; decline trial; spray volume 3738 l/ha; equipment and weather conditions not stated; soil not stated; samples from 2 x 3 replicate field plots (Gibson Ranch and Harrison Ranch). Gibson Ranch and Harrison Ranch are considered as the same test location. Harvest May/Jun 85; each treatment was conducted on one tree only; sample size not stated. Storage 25-32 days (temperature not stated).

Pome fruits (group 002)

Results of trials on apples are shown in Table 118 and on pears in Table 119.

In most reports of non-GLP trials on apples and pears in South Africa in 1976/77, Canada in 1983 and 1984, Germany in 1975, 1976, 1978 and 1979, the UK in 1975, 1977, 1978 and 1982, The Netherlands in 1974, 1975, 1976 and 1979, France in 1974, 1975, 1976 and 1979, Italy in 1974, 1975, 1976, 1982, 1985 and Spain in 1974, 1975 and 1976 (Buisman, 1980, 1981, 1983, 1984; Buisman and Snijders, 1987a,c; Buisman and Verhaar, 1985a; Buisman *et al.*, 1975, 1976b-f; De Wilde, 1977a-h, 1978c, 1979b, 1980, 1982, 1984; De Wilde and Buisman, 1985a,b) the field, sampling and storage conditions were not reported. The samples were analysed for diflubenzuron by method LAI 3-86-2. Residues were not corrected for concurrent method recoveries (29%-160%) or matrix interferences (max. 0.16 mg/kg for pears) (Table 48). Results at or below 0.5 mg/kg diflubenzuron are not considered valid (matrix interferences and low recoveries); they are considered valid in the range 0.6-1.0 mg/kg diflubenzuron. Results from the 1974 French and Italian trials (Buisman *et al.*, 1976e), the 1976 German trials (De Wilde, 1977e) and the 1976 Italian and Spanish trials (De Wilde, 1977h) could not be evaluated because of high values in control samples (0.34, 0.89 and 1.0 mg/kg respectively). Results from the 1975 Dutch trials on pears (De Wilde, 1977b) could not be evaluated because of high method recoveries at levels between 1 and 2 mg/kg diflubenzuron (121%-129%).

In non-GLP trials on apples in Italy in 1987 (Pouwelse, 1989) samples were analysed for diflubenzuron by method LAI 3-86-14. Residues were not corrected for storage stability (67%-92% at 0.1-1 mg/kg diflubenzuron) or matrix interferences (max. 0.028 mg/kg diflubenzuron for apples) (Table 56). Because of matrix interferences results below 0.1 mg/kg are not considered valid.

Samples from GLP trials on apples in Germany in 1993 (Thus and Allan, 1995, 1996) were analysed for diflubenzuron by HPLC method RES060. Residues were not corrected for storage stability (74%-97% at 0.1-1 mg/kg diflubenzuron), concurrent method recoveries (70%-103%) or matrix interferences (max. 0.011 mg/kg diflubenzuron, Table 58). Because of matrix interferences results below 0.04 mg/kg are not considered valid.

In non-GLP trials on apples in Poland in 1994 (Nowacki, 1995) samples were analysed for diflubenzuron by the Polish HPLC method. Residues were not corrected for concurrent method recoveries (82%-84%) or matrix interferences (<0.0025 mg/kg = LOQ, Table 59).

In non-GLP trials on apples in Japan in 1976 (Goto, 1977) analyses were carried out on both whole fruit (Goto, 1977) and the edible parts (Nakayama, 1977). Because the study report does not state what is meant by the edible part of an apple, only the results for the whole fruit are shown in the Table. Whole fruit samples were analysed for diflubenzuron by the Japanese HPLC method I. Residues were not corrected for concurrent method recoveries (68%-93%) or matrix interferences (max. 0.014 mg/kg diflubenzuron, Table 50). Because of matrix interferences, results below 0.05 mg/kg are not considered valid.

Samples from trials on apples and pears in the USA in 1983 (ABC, 1984, non-GLP) were analysed for diflubenzuron by HPLC method RES010. Residues were corrected for concurrent method recoveries (98%-136% for apples and 100% for pears), but not for matrix interferences (<0.05 mg/kg diflubenzuron, Table 52). Because of high recoveries (136%), results below 0.5 mg/kg are not considered valid.

In trials on apples and pears in the USA in 1984 (Duphar, 1985a, non-GLP) samples were analysed for diflubenzuron by GC-ECD method LAI 3-86-6. Residues were not corrected for concurrent method recoveries (65%-88%) or matrix interferences (<0.05 mg/kg diflubenzuron, Table 74). Because of low recoveries, results below 1.0 mg/kg are not considered valid.

In trials on apples and pears in the USA in 1986 (Ball, 1987, non-GLP) residues were corrected for concurrent method recoveries (74%-90%), but not for matrix interferences (<0.05 mg/kg diflubenzuron).

Samples from trials on pears in the USA in 1996 (Gaydosh and Rose, 1999, GLP) and 1997 (Dorschner and Gaydosh 2000, GLP) were analysed by GC-ECD method LAI 3-86-6 for diflubenzuron, GC-ECD LAI 3-86-9 for CPU and PTRL 645W for PCA. Residues were not corrected for concurrent method recoveries (62%-114% for diflubenzuron, 65%-115% for CPU, 87%-127% for PCA) or matrix interferences (<0.05 mg/kg diflubenzuron, max 0.002 mg/kg CPU, <0.005 mg/kg PCA) (Table 74 for diflubenzuron, Table 83 for CPU and Table 93 for PCA). Pears collected from the Ontario site contained substantially higher residues, attributed to dormant oil (2%) being mistakenly added to all four applications. Results for PCA were <0.005 mg/kg in all samples and are not tabulated. Because storage times for pears (159-286 days for CPU and 138-298 days for PCA) exceeded the stability periods (up to 3 months for CPU, 1-2 weeks for PCA), the CPU and PCA results are not considered valid.

Table 118. Residues of diflubenzuron in whole apples.

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	Ref
single applications							
Netherlands, Leunen, 1975, Golden Delicious	WP 250	ns	1x0.010	-	159	<0.03	3
Netherlands, ‘s-Graveland, 1975, Golden Delicious	WP 250	ns	1x0.010	-	157	<0.03	3
Netherlands, Helden, 1975, Winston	WP 250	ns	1x0.010	-	157	<0.03	3
Netherlands, Cothen, 1975, Golden Delicious	WP 250	ns	1x0.010	-	117	<0.03	3
Netherlands, ‘s-Graveland, 1975, Golden Delicious	WP 250	ns	1x0.010	-	104	<0.03	3
Netherlands, Lienden, 1974, James Grieve	WP 250	ns	1x0.012	-	18 32 44	0.29 0.42 0.17[4]	4

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	Ref
Netherlands, Leunen, 1975, Golden Delicious	WP 250	ns	1x0.020	-	159	<0.03	[3]
Netherlands, ‘s-Graveland, 1975, Golden Delicious	WP 250	ns	1x0.020	-	157	<0.03	[3]
Netherlands, Helden, 1975, Winston	WP 250	ns	1x0.020	-	157	<0.03	[3]
Netherlands, Cothen, 1975, Golden Delicious	WP 250	ns	1x0.020	-	117	<0.03	[3]
Netherlands, ‘s-Graveland, 1975, Golden Delicious	WP 250	ns	1x0.020	-	114	<0.03	[3]
Netherlands, ‘s-Graveland, 1975, Golden Delicious	WP 250	ns	1x0.020	-	104	0.060	[3]
Netherlands, Geldermalsen, 1975, Winston	WP 250	ns	1x0.020	-	77	0.092	[3]
Netherlands, Giessen, 1975, Golden Delicious	WP 250	ns	1x0.020	-	74	0.096	[3]
Netherlands, Geldermalsen, 1975, Cox Orange	WP 250	ns	1x0.020	-	56	0.12	[3]
Netherlands, Tricht, 1975, Boskoop	WP 250	ns	1x0.020	-	56	0.076	[3]
Netherlands, Tricht, 1975, Cox Orange	WP 250	ns	1x0.020	-	56	<0.03	[3]
Netherlands, Tricht, 1975, Ingrid Marie	WP 250	ns	1x0.020	-	56	0.064	[3]
Netherlands, Zaltbommel, 1975, Cox Orange	WP 250	ns	1x0.020	-	53	0.052	[3]
Netherlands, Giessen, 1975, Cox Orange	WP 250	ns	1x0.020	-	53	0.10	[3]
Netherlands, Thedingsweert, 1975, Boskoop	WP 250	ns	1x0.020	-	53	0.10	[3]
Netherlands, Giessen, 1975, Boskoop	WP 250	ns	1x0.020	-	53	0.17	[3]
Netherlands, Ingen, 1975, Cox Orange	WP 250	ns	1x0.020	-	53	0.072	[3]
Netherlands, Marknesse, 1974, James Grieve	WP 250	ns	1x0.020	-	0 8 15 28 28[4]	0.28[4] 0.22[4] 0.17[4] 0.16[4] 0.16[4]	[4]
Netherlands, ‘s-Graveland, 1974, Golden Delicious	WP 250	ns	1x0.020	-	0 14 28 42 57 71	0.62[4] 0.26[4] 0.40[4] 0.23[4] 0.22[4] 0.34[4]	[4]
Netherlands, Kapelle-Biezelinge, 1974, Golden Delicious IX	WP 250	ns	1x0.020	-	0 15 28 42 70	0.53[4] 0.31[4] 0.25[4] 0.28[4] 0.15[4]	[4]
Netherlands, Lienden, 1974, James Grieve	WP 250	ns	1x0.025	-	18 32 44	0.67 0.44 0.34	[4]
Netherlands, Twello, 1975, Golden Delicious	WP 250	ns	1x0.030	-	167	<0.03	[3]
Netherlands, Sevenum, 1975, Jonathan	WP 250	ns	1x0.030	-	167	<0.03	[3]
Netherlands, Borsele, 1975, Cox’s Orange Pippin	WP 250	ns	1x0.030	-	139	0.019[4]	[4]
Netherlands, ‘s-Graveland, 1975, Golden Delicious	WP 250	ns	1x0.030	-	114	<0.03	[3]
Netherlands, ‘s-Graveland, 1975, Golden Delicious	WP 250	ns	1x0.030	-	104	0.10	[3]

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	Ref
Netherlands, Leunen, 1975, Golden Delicious	WP 250	ns	1x0.040	-	159	<0.03	3
Netherlands, 's-Graveland, 1975, Golden Delicious	WP 250	ns	1x0.040	-	157	<0.03	3
Netherlands, Helden, 1975, Winston	WP 250	ns	1x0.040	-	157	<0.03	3
Netherlands, Cothen, 1975, Golden Delicious	WP 250	ns	1x0.040	-	117	<0.03	3
Netherlands, Leunen, 1975, Golden Delicious	WP 250	ns	1x0.040	-	111	0.048	3
Netherlands, 's-Graveland, 1975, Golden Delicious	WP 250	ns	1x0.040	-	102	<0.03	3
Netherlands, Dronten, 1975, James Grieve	WP 250	ns	1x0.040	-	24	0.15	3
Germany, Angermund, 1975, Golden Delicious	WP 250	1x0.30	1x0.015	-	0 21 35 49 56 70	0.33 0.31 0.26 0.23 0.012 0.17	5
Germany, Angermund, 1975, James Grieve	WP 250	1x0.30	1x0.015	-	0 14 21 28 35 42	0.42 0.14 0.088 0.10 0.10 0.064	5
Germany, Angermund, 1975, Golden Delicious	WP 250	1x0.60	1x0.030	-	0 21 35 49 56 70	0.96 0.62 0.41 0.41 0.000 0.29	5
Germany, Angermund, 1975, James Grieve	WP 250	1x0.60	1x0.030	-	0 14 21 28 35 42	0.70 0.22 0.20 0.16 0.12 0.12	5
UK, Benenden, Kent, 1978, Cox Orange P.	WP 250	1x0.15	ns	-	13 20 27	0.17 0.15 0.19	6
UK, Cranbrook, Kent, 1978, Bramley	WP 250	1x0.15	ns	-	9 16 23	0.21 0.23 0.14	6
UK, Tenterden, Kent, 1978, Egremont Russet	WP 250	1x0.15	ns	-	13 20 27	0.26 0.23 0.18	6
UK, Larkin, Horsmonden, Kent, 1977, Bramley	WP 250	1x0.25	ns	-	81	0.023	7
UK, Larkin, Horsmonden, Kent, 1977, Newton	WP 250	1x0.25	ns	-	81	0.015	7
UK, Glover, Brenchley, Kent, 1977, Ida Red	WP 250	1x0.25	ns	-	43	0.12	7
UK, Glover, Brenchley, Kent, 1977, Golden Delicious	WP 250	1x0.25	ns	-	43	0.20	7
UK, Glover, Brenchley, Kent, 1977, Mackintosh	WP 250	1x0.25	ns	-	43	0.17	7
UK, Glover, Horsmonden, Kent, 1977, Bramley	WP 250	1x0.25	ns	-	43	0.19	7
UK, Benenden, Kent, 1978, Cox Orange P	WP 250	1x0.25	ns	-	13 20 27	0.081 <0.01 0.21	6

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	Ref
UK, Cranbrook, Kent, 1978, Bramley	WP 250	1x0.25	ns	-	9	0.17	6
					16	0.028	
					23	0.18	
UK, Tenterden, Kent, 1978, Egremont Russet	WP 250	1x0.25	ns	-	13	0.17	6
					20	0.029	
					27	0.19	
Poland, Dabrowice, 1994, Lobo	WP 250	1x0.10	1x0.010	-	0	0.099	8
					4	0.089	
					7	0.067	
					14	0.043	
					21	0.029	
					28	0.026	
Italy, Magre (BZ), 1982, Jonathan	WP 050	ns	1x0.0075	-	21	0.18	8a
Italy, Asti, 1982, Starking	WP 050	ns	1x0.012	-	15	0.22	8a
Italy, Frangarto (BZ), 1985, Golden Delicious	WP 250	1x0.012	1x0.012	-	0	0.50	9
					13	0.22	
					19	0.23	
					26	0.15	
					33	0.21	
					42	0.19	
Italy, Bolzano, 1987, Jonathan	WP 050	1x0.22	1x0.012	-	0	0.83	10
					7	0.44	
					11	0.28	
					25	0.34	
					42	0.29	
Italy, Isola, 1987, Golden Delicious	WP 050	1x0.25	1x0.012	-	0	0.34	10
					7	0.19	
					15	0.21	
					31	0.15	
					46	0.12	
Italy, Appiano, 1987, Kalterer	WP 050	1x0.31	1x0.012	-	0	1.1	10
					4	1.0	
					11	0.66	
					25	0.65	
					42	0.27	
Italy, Egna, 1975, Golden Delicious	WP 250	ns	1x0.015	-	91	0.14	17
S. Africa, Houwhoek, Western Cape, 1977, -	WP 250	ns	1x0.020	-	1	0.38	11
					2	0.68	
					4	0.27	
					8	0.13	
					16	0.13	
					32	0.20	
S. Africa, Elgin, Western Cape, 1977, -	WP 250	ns	1x0.020	-	1	0.56	11
					2	0.74	
					4	0.44	
					8	0.54	
					16	0.29	
					32	0.26	
S. Africa, Houwhoek, Western Cape, 1977, -	WP 250	ns	1x0.040	-	1	0.70	11
					2	0.10	
					4	0.81	
					8	0.34	
					16	0.33	
					32	0.23	
S. Africa, Elgin, Western Cape, 1977, -	WP 250	ns	1x0.040	-	1	1.4	11
					2	0.64	
					4	1.0	
					8	1.2	
					16	1.1	
					32	0.63	

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	Ref
Japan, Iwate, 1976, Star King Delicious	WP 250	1x1.2	1x0.025	-	30 60	<0.008 (2), mean <0.008 0.040, 0.043, mean 0.042	12
Japan, Nagano, 1976, Red Ball	WP 250	1x1.8	1x0.025	-	30 60	0.052, 0.076, mean 0.064 0.12, 0.10, mean 0.11	12
Canada, Nova Scotia, 1984, Courtland	WP 250	1x0.34	1x0.010	-	42 71	0.11 0.061	13
Canada, Nova Scotia, 1984, Courtland	WP 250	1x0.44	1x0.013	-	42 71	0.15 0.13	13
two applications							
Netherlands, Lienden, 1974, James Grieve	WP 250	ns	2x0.012	17	1 8 15 27	1.3 0.91 0.80 0.55	4
Netherlands, Tolbert, 1975, Golden Delicious	WP 250	ns	2x0.020	22	145	<0.03	3
Netherlands, ‘s-Graveland, 1975, Golden Delicious	WP 250	ns	2x0.020	31	127	0.12	3
Netherlands, Zevenbergschehoek, 1976, Golden Delicious	WP 250	ns	2x0.020	40	0[1] 40[1] 0 31 49 71 85 95	1.0 0.10 0.45 0.18 0.17 0.17 0.15 0.10	14
Netherlands, ‘s-Graveland, 1976, Cox’s Orange Pippin	WP 250	ns	2x0.020	34	0[1] 34[1] 0 21 30 42 56 67	0.55 0.14 0.51 0.18 0.11 0.12 0.14 0.19	14
Netherlands, Wessem, 1975, Cox Orange	WP 250	ns	2x0.020	ns	50	0.088	3
Netherlands, ‘s-Graveland, 1974, Golden Delicious	WP 250	ns	2x0.025	14	35	0.44[4]	4
Netherlands, Zevenbergse Hoek, 1974, Golden Delicious	WP 250	ns	2x0.025	15	30	0.42[4]	4
Netherlands, Lienden, 1974, James Grieve	WP 250	ns	2x0.025	17	1 8 15 27	1.1 1.6 0.63 0.89	4
Netherlands, Lewedorp, 1974, James Grieve	WP 250	ns	2x0.030	19	29 50	0.31[4], 0.34[4] 0.31	4
Netherlands, Lewedorp, 1974, Golden Delicious	WP 250	ns	2x0.030	19	107	0.36	4
Netherlands, Zevenbergse Hoek, 1974, Golden Delicious	WP 250	ns	2x0.030	15	30 104	0.47[4] 0.42	4
Netherlands, Leunen, 1974, Golden Delicious	WP 250	ns	2x0.030	19	95	0.35	4
Netherlands, ‘s-Graveland, 1974, Golden Delicious	WP 250	ns	2x0.030	14	35	0.41[4]	4
Netherlands, Tolbert, 1975, Golden Delicious	WP 250	ns	2x0.040	22	145	0.044	3
Netherlands, ‘s-Graveland, 1975, Golden Delicious	WP 250	ns	2x0.040	31	127	0.044	3
Netherlands, Lewedorp, 1975, Golden Delicious	WP 250	ns	2x0.040	43	113	0.088	3

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	Ref
Netherlands, Sevenum, 1975, Jonathan	WP 250	ns	2x0.040	26	111	0.056	3
UK, Firkin, Teddington, Glouc, 1977, Bramley.	WP 250	2x0.25	ns	27	52	0.13	7
UK, Firkin, Teddington, Glouc, 1977, Warner.	WP 250	2x0.25	ns	27	52	0.16	7
UK, Firkin, Teddington, Glouc, 1977, Worcester	WP 250	2x0.25	ns	27	52	0.15	7
UK, Seven Oaks, 1975, Cox Orange P.	WP 250	1x0.38 1x0.68	1x0.067 1x0.11	67	80	0.028	15
UK, Lymington, 1975, Cox Orange P.	WP 250	2x0.70	ns	21	74	0.13	15
Germany, 7124 Bonnigheim, 1976, Golden Delicious	WP 250	2x0.40	2x0.020;	22	0[1]	0.58, 0.82, mean 0.70	16
					22[1]	0.24, 0.30, mean 0.27	
					0	1.2 (2), mean 1.2	
					13	0.65	
					28	0.50, 0.51 mean 0.51	
					41	0.56	
					54	0.51	
					67	0.35	
Germany, 7144 Asperg, 1976, Cox Orange P.	WP 250	2x0.40	2x0.020	22	0[1]	1.4, 1.5, mean 1.5	16
					22[1]	0.37, 0.46, mean 0.41	
					0	1.8 (2), mean 1.8	
					13	1.2	
					28	0.76, 1.0 mean 0.89	
					41	0.48	
					54	0.24	
Germany, 5301 Impekoven, 1976, Cox Orange P.	WP 250	2x0.40	2x0.020	21	0[1]	0.21, 0.35 mean 0.28	16
					21[1]	0.060, 0.082, mean 0.071	
					0	0.43, 0.62, mean 0.52	
					17	0.25	
					28	0.21, 0.27 mean 0.24	
					42	0.19	
					56	0.14	
Italy, Altedo, 1975, Golden Delicious	WP 250	ns	2x0.012	31	97	0.16	17
Italy, Baricelle, 1975, Sterk Krims	WP 250	ns	2x0.012	58	74	0.064	17
Italy, Egna, 1975, Golden Delicious	WP 250	ns	2x0.015	52	91	0.044	17
Italy, Egna, 1975, Golden Delicious	WP 250	ns	2x0.015	49	42	0.12	17
Italy, Bolzano, 1974, Gravestein	WP 250	ns	2x0.020	53	49	0.25	18
Italy, Firenze, 1976, Stark Delicious and Golden Delcious	WP 250	ns	1x0.025; 1x0.020	62	38	0.18, 0.19; mean 0.18;	19
					50	0.18, 0.27; mean 0.22; 0.19, 0.21; mean 0.20	

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	Ref
Italy, Altedo, 1975, Golden Delicious	WP 250	ns	2x0.025	31	97	0.072	17
Italy, Egna, 1975, Golden Delicious	WP 250	ns	2x0.025	52	91	0.15	17
Italy, Baricelle, 1975, Sterk Krims	WP 250	ns	2x0.025	58	74	0.11	17
Italy, Bolzano, 1974, Gravestein	WP 250	ns	2x0.040	53	49	0.42	18
Canada, Summerland Station (BC), 1983, Spartan	WP 250	1x0.25[#] 1x0.25	1x0.010[#] 1x0.010	58	33	0.10, 0.11, 0.18, mean 0.13	20
					60	0.12 (3), mean 0.12	
Canada, Vineland Station (Ont.), 1984, Mac Intosh	WP 250	2x0.18	ns	63	24	0.095, 0.098, 0.13, 0.18, mean 0.13	13
					54	0.085, 0.096, 0.14, 0.19, mean 0.12	
Canada, Smithfield Station (Ont.), 1984, Mac Intosh	WP 250	1x0.44; 1x0.34	ns	36	59	0.22	13
					92	<0.03 (3), mean <0.03	
three applications							
Netherlands, Twello, 1979, Cox's Orange P.	WP 250	ns	3x0.010	29, 42	13	0.38	21
				29, 24	31	0.36	
				29, 42	23	0.25	
				29, 24	41	0.29	
Netherlands, 's-Graveland, 1979, James Grieve	WP 250	ns	3x0.010	31, 36	14	0.22	21
				31, 22	28	0.15	
				31, 36	31	0.15	
				31, 22	45	0.12	
Netherlands, 's-Graveland, 1979, Golden Delicious	WP 250	ns	3x0.010	31, 45	14	0.37	21
				31, 31	28	0.43	
Netherlands, Zevenbergsche Hoek, 1976, Golden Delicious	WP 250	ns	2x0.020; 1x0.015	40, 31	0[1]	1.0	14
					40[1]	0.10	
					0[2]	0.45	
					31[2]	0.18	
					0	0.42	
					14	0.38	
					28	0.30	
					40	0.28	
					54	0.17	
					64	0.26	
Netherlands, 's-Graveland, 1976, Cox's Orange Pippin	WP 250	ns	2x0.020; 1x0.015	34, 30	0[1]	0.55	14
					34[1]	0.14	
					0[2]	0.51	
					30[2]	0.11	
					0	0.28	
					7	0.28	
					14	0.26	
					28	0.27	
					37	0.22	
UK, Bekesbourne, 1975, Cox Orange P.	WP 250	1x0.25; 2x0.38	1x0.028; 2x0.042	70, 19	55	0.42	15
UK, Hannington, 1975, Cox Orange P.	WP 250	1x0.25; 2x0.38	1x0.036; 2x0.054	77, 20	54	0.57	15
UK, Bekesbourne, 1975, Cox Orange P.	WP 250	1x0.38; 2x0.50	1x0.042; 2x0.056	70, 19	55	0.72	15
UK, Hannington, 1975, Cox Orange P.	WP 250	1x0.38; 2x0.50	1x0.054; 2x0.071	77, 20	54	1.7	15
UK, Bekesbourne, 1975, Cox Orange P.	WP 250	1x0.50; 2x0.62	1x0.056; 2x0.069	70, 19	55	0.89	15
UK, Hannington, 1975, Cox Orange P.	WP 250	1x0.50; 2x0.62	1x0.071; 2x0.089	77, 20	54	1.3	15

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	Ref
Germany, 7144 Asperg, 1976, Cox Orange P.	WP 250	2x0.40; 1x0.30	2x0.020; 1x0.015	22, 28	0[1]	1.4, 1.5, mean 1.5	16
					22[1]	0.37, 0.46, mean 0.41	
					0[2]	1.8 (2), mean 1.8	
					28[2]	0.76, 1.0 mean 0.89	
					0	0.80	
					12	0.92	
					19	0.72	
					26	0.68	
					33	0.89	
Germany, 5301 Impekoven, 1976, Cox Orange P.	WP 250	2x0.40; 1x0.30	2x0.020; 1x0.015	21, 28	0[1]	0.21, 0.35 mean 0.28	16
					21[1]	0.060, 0.082, mean 0.071	
					0[2]	0.43, 0.62, mean 0.52	
					28[2]	0.21, 0.27 mean 0.24	
					0	0.51	
					14	0.41	
					21	0.38	
					28	0.25	
					35	0.44	
Germany, 7124 Bonnigheim, 1976, Golden Delicious	WP 250	3x0.40	3x0.020	22, 28	0[1]	0.58, 0.82, mean 0.70	16
					22[1]	0.24, 0.30, mean 0.27	
					0[2]	1.2 (2), mean 1.2	
					28[2]	0.50, 0.51 mean 0.51	
					0	1.2	
					12	0.84	
					26	0.49	
					39	0.62	
					54	0.59	
N. France, Parcay les pins (49), 1976, Golden Delicious	WP 250	ns	3x0.010	32, 31	26	0.29	22
N. France, Parcay les pins (49), 1976, Golden Delicious	SC 480?	ns	3x0.019	32, 31	26	0.46	22
S. France, EUS (66), 1976, Golden Delicious	WP 250	ns	3x0.019	30, 27	31	0.43	22
S. France, La Celle, 1976, Golden Delicious	WP 250	ns	3x0.019	29, 35	40	0.42	22
Italy, St. Gabriele (BO), 1974, Golden Delicious	WP 250	ns	3x0.010	10, 37	56	0.31	18
Italy, S. Gabrielle (BO), 1975, Golden Delicious	WP 250	ns	3x0.010	57, 32	51	0.31	17
Italy, Altedo, 1975, Golden Delicious	WP 250	ns	3x0.012	31, 32	65	0.048	17
Italy, Baricelle, 1975, Sterk Krims	WP 250	ns	3x0.012	58, 34	40	0.13	17
Italy, Egna, 1975, Golden Delicious	WP 250	ns	3x0.015	52, 49	42	0.47	17
Italy, Sesto Imolese, 1975, Steymen	WP 250	ns	3x0.020	58, 32	64	0.41	17
Italy, St. Gabriele (BO), 1974, Golden Delicious	WP 250	ns	3x0.020	10, 37	56	0.59	18
Italy, Bolzano, 1974, Golden Delicious	WP 250	ns	3x0.020	57, 56	31	0.59	18

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	Ref
Italy, Altedo, 1975, Golden Delicious	WP 250	ns	3x0.025	31, 32	65	0.29	17
Italy, Egna, 1975, Golden Delicious	WP 250	ns	3x0.025	52, 49	42	0.57	17
Italy, Baricelle, 1975, Sterk Krims	WP 250	ns	3x0.025	58, 34	40	0.31	17
Italy, St. Gabriele (BO), 1974, Golden Delicious	WP 250	ns	3x0.030	10, 37	56	1.1	18
Italy, Bolzano, 1974, Golden Delicious	WP 250	ns	3x0.040	57, 56	31	0.90	18
Spain, Torrebaja, Valencia, 1976, Golden Delicious	WP 250	ns	3x0.015	27, 27	71	0.78	19
Japan, Iwate, 1976, Star King Delicious	WP 250	3x1.2	3x0.025	14, 14	60 90	0.42, 0.38; mean 0.40 0.19, 0.15; mean 0.17	12
Japan, Nagano, 1976, Red Ball	WP 250	3x1.8	3x0.025	14, 14	60	0.27, 0.20; mean 0.23	12
USA, Watsonville, CA, 1983, Red Delicious	WP 250	3x0.21	ns	56, 47	28	0.18	23
USA, Watsonville, CA, 1983, Red Delicious	WP 250	3x0.42	ns	56, 47	28	0.86	23
USA, Watsonville, CA, 1983, Newton	WP 250	3x0.21	ns	56, 47	28	0.31	23
USA, Watsonville, CA, 1983, Newton	WP 250	3x0.42	ns	56, 47	28	0.51	23
USA, Hood River, OR, 1983, Red Delicious	WP 250	3x0.21	ns	33, 63	28	0.34	23
USA, Hood River, OR, 1983, Red Delicious	WP 250	3x0.42	ns	33, 63	28	0.64	23
USA, Wapato, WA, 1983, Red Delicious	WP 250	3x0.42	ns	29, 63	28	0.37	23
USA, Wapato, WA, 1984, Granny Smith	WP 250	3x0.28	3x0.037	47, 48	28	0.20	24
USA, Zillah, WA, 1984, Red Delicious	WP 250	3x0.28	3x0.020	29, 47	28	0.21	24
USA, Hood River, OR, 1984, Red Delicious	WP 250	3x0.28	3x0.0075	19, 48	28	0.14	24
USA, Wenatchee, WA, 1984, McIntosh	WP 250	3x0.28	3x0.0075	26, 48	28	0.21	24
USA, Bennington, VT, 1986, Red Delicious	WP 250	3x0.28	3x0.0075	16, 93	11 28	0.57, 0.58, mean 0.58 0.57, 0.60, mean 0.58	25
USA, Westford, MA, 1986, McIntosh	WP 250	3x0.28	3x0.0075	26, 95	28	0.08	25
USA, Sodus, NY, 1986, Greening	WP 250	3x0.28	3x0.0075	17, 78	28	<0.05 or 0.58? mislabelled?	25
USA, Fennville, MI, 1986, Golden Delicious	WP 250	3x0.28	3x0.020	21, 103	14 28	0.15 0.23	25
USA, Marine, IL, 1986, Granny Smith	WP 250	3x0.28	3x0.030	10, 29	37	0.60	25
USA, Marine, IL, 1986, Spartan	WP 250	3x0.28	3x0.030	10, 10	29	0.56	25
USA, Highland, NY, 1986, McIntosh	WP 250	3x0.28	3x0.0075	18, 36	14 28	0.18 0.16	25
USA, Highland, NY, 1986, Red Delicious	WP 250	3x0.28	3x0.0075	18, 36	28	0.14	25
Canada, Jordan Exp.Farm, (Ont), 1983, Mac Intosh	WP 250	3x 0.19	3x0.030	17, 61	27 62	0.17 0.13	26
four applications							

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	Ref
Germany, 47918 Tönisvorst 1993, Elstar	WP 250	1x0.30; 3x0.18	1x0.020; 3x0.012	17, 64, 16	0 7 14 28 35	0.49 0.37 0.52 0.38 0.48	27
Germany, 53340 Ersdorf 1993, James Grieve	WP 250	1x0.30; 3x0.18	1x0.020; 3x0.012	20, 18, 20	0 7 14 28 35	0.62 0.44 0.45 0.29 0.30	27
Germany, 47918 Tönisvorst 1993, Elstar	WG 800	1x0.30; 3x0.18	1x0.020; 3x0.012	17, 64, 16	0 7 14 28 35	0.41 0.41 0.52 0.41 0.37	27
Germany, 53340 Ersdorf 1993, James Grieve	WG 800	1x0.30; 3x0.18	1x0.020; 3x0.012	20, 18, 20	0 7 14 28 35	0.64 0.52 0.43 0.36 0.31	27
Germany, 74366 Kirchheim 1993, Jonagold	WP 250	1x0.30; 3x0.18	1x0.060; 3x0.036	32, 33, 31	0 7 14 28 35	0.12 0.18 0.16 0.12 0.095	27
Germany, 78359 Orsingen-Nenzingen, 1993, Idared	WP 250	1x0.30; 3x0.18	1x0.060; 3x0.036	6, 33, 36	0 7 14 28 35	0.63 0.33 0.39 0.37 0.34	27
Germany, 74366 Kirchheim 1993, Jonagold	WG 800	1x0.30; 3x0.18	1x0.060; 3x0.036	32, 33, 31	0 7 14 28 35	0.18 0.21 0.16 0.11 0.12	27
Germany, 78359 Orsingen-Nenzingen, 1993, Idared	WG 800	1x0.30; 3x0.18	1x0.060; 3x0.036	6, 33, 36	0 7 14 28 35	0.46 0.35 0.32 0.30 0.33	27
Germany, 5301 Impekhofen, 1979, James Grieve	WP 250	3x0.30; 1x0.22	3x0.019; 1x0.014	21, 14, 21	0 7 14 21 28	0.80 0.83 0.95 0.73 0.66	28
Germany, Stockach, 1978, Alkmene	WP 250	3x0.30; 1x0.22	3x0.040; 1x0.030	13, 16, 20	0 9 14 21 28	1.1 0.72 0.69 0.62 0.63	29
Germany, 6714 Hochstadt, 1979, Oldenburg	WP 250	3x0.30; 1x0.22	3x0.060; 1x0.045	7, 7, 7	0 7 14 21 29	1.1 1.2 1.0 1.0 0.84	28
Germany, 5301 Impekhofen, 1978, James Grieve	WP 250	3x0.90; 1x0.68	3x0.18; 1x0.14	21, 14, 21	0 7 14 21 29	2.4 2.0 1.9 1.8 1.8	29
S. France, Dinel Hauterive (47) 1979, Golden Delicious	WP 250	2x0.12; 2x0.10	ns	40, 28, 28	14	0.20	30
N. France, Pruniers (49) 1979, Golden Delicious	WP 250	ns	4x0.010	23, 25, 60	14	0.15	30

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	Ref
S. France, St. Michel d'Euzet (30), 1979, Golden Delicious	WP 250	ns	4x0.010	20, 38, 29	16	0.19	30
S. France, Estillac (47) 1979, Golden Delicious	WP 250	ns	4x0.010	21, 38, 25	17	0.34	30
S. France, Estillac (47) 1979, Golden Delicious	WP 250	ns	4x0.019	21, 21, 28	31	0.60	30
S. France, St. Michel d'Euzet (30), 1979, Golden Delicious	WP 250	ns	4x0.019	20, 20, 30	33	3.6	30
S. France, Astaffort (47), 1975, Golden Delicious	WP 250	ns	4x0.025	21, 28, 28	ns	0.30	15
Italy, Terlano (BZ), 1976, Jonathan	WP 250	ns	4x0.012	35, 22, 41	58	0.14	19
Italy, Terlano (BZ), 1976, Jonathan	WP 250	ns	4x0.025	35, 22, 41	58	0.17	19
Spain, Torrebaja, Valencia, 1976, Golden Delicious	WP 250	ns	4x0.015	27, 27, 35	36	1.1	19
South Africa, Beaucoup deLeau, Franschhoek, 1976/77, -	WP 250	ns	1x0.025; 3x0.020	28, 26, 38	22	1.1	11
USA, Hood River, (OR), 1983, Red Delicious	WP 250	4x0.21	ns	29, 26, 27	29	0.81	23
USA, Wapato, WA, 1984, Granny Smith	WP 250	4x0.28	4x0.037	75, 48, 14	14	0.34	24
USA, Zillah, WA, 1984, Red Delicious	WP 250	4x0.28	4x0.020	29, 47, 14	14	0.40	24
USA, Hood River, OR, 1984, Red Delicious	WP 250	4x0.28	4x0.0075	22, 36, 13	14	0.27	24
USA, Wenatchee, WA, 1984, McIntosh	WP 250	4x0.28	4x0.0075	26, 48, 14	14	0.28	24
five applications							
S. France, EUS (66), 1975, Golden Delicious	WP 250	ns	5x0.015	28, 14, 21, 21	36	0.28	15
S. France, Avignon (84), 1976, Golden Delicious	WP 250	ns	5x0.020	15, 14, 16, 16	60	0.26	22
S. France, EUS (66), 1975, Golden Delicious	WP 250	ns	5x0.025	28, 14, 21, 21	36	0.76	15
Italy, Altedo (BO), 1974, Golden Delicious	WP 250	ns	5x0.020	40, 29, 15, 33	56	0.80	18
Italy, Baricello (BO), 1974, Golden Delicious	WP 250	ns	5x0.020	34, 30, 14, 28	55	0.90	18
Italy, Villanora d'Aida (PC), 1976, Golden Delicious	WP 250	ns	5x0.020	23, 15, 27, 14	17	0.27	19
Italy, Altedo (BO), 1974, Golden Delicious	WP 250	ns	5x0.040	40, 29, 15, 33	56	1.4	18
Italy, Baricello (BO), 1974, Golden Delicious	WP 250	ns	5x0.040	34, 30, 14, 28	55	1.5	18
Spain, Valencia, 1975, Golden Delicious	WP 250	ns	5x0.010	26, 30, 28, 15	35	0.49	15
Spain, Valencia, 1975, Golden Delicious	WP 250	ns	5x0.015	26, 30, 28, 15	35	0.92	15
Spain, Torrebaja, Valencia, 1976, Golden Delicious	WP 250	ns	5x0.015	27, 27, 35, 22	14	0.73	19
sixfold applications							
N. France, Pruniers (49), 1974, Golden Delicious	WP 250	3x0.30; 3x0.28	6x0.025	14, 17, 12, 14, 15	12	1.2	18
N. France, Pruniers (49), 1974, Golden Delicious	WP 250	3x0.48; 3x0.44	6x0.040	14, 17, 12, 14, 15	12	1.8	18

including 500 mg/l Tween

[1] 1976 Dutch and German apple trials: days after first treatment

[2] 1976 Dutch and German apple trials: days after second treatment

[3] DI-1249, Buisman *et al.*, 1976b, non-GLP, harvest Aug/Sept/Oct, equipment, soil, weather conditions, plot size and sampling not stated, storage at –20°C (storage time not stated).

[4] DI-2984, Buisman *et al.*, 1975, non-GLP, mainly decline trials (same last treatment date, different harvest times), equipment and soil not stated, harvest July/Aug/Sept/Oct, rainfall in Sept and Oct was above normal, sampling at random, sample weight 6.6-15.4 kg; 4-5 apples per tree, plot size 12-20 trees, storage at –20°C (storage time not stated). Results marked [4] were the means of duplicate analyses. Marknesse: PHI was 28 days after treatment with storage for 14 days (storage conditions not stated). The treatment in Borsele was pre-bloom. Samples from Lewedorp (James Grieve, 29 days PHI) were assumed to be from duplicate trials (not stated) and each sample was analysed in duplicate.

[5] DI-2403, Buisman *et al.*, 1976d, non-GLP, decline trials (same last treatment date, different harvest times), spray volume 2000 l/ha, equipment, soil, weather conditions, plot size and sampling not stated, harvest Aug/Sept/Oct, storage at –25°C (storage time not stated).

[6] DI-201, De Wilde, 1980, non-GLP; spray by high volume hand lance; decline trials (same harvest date for different PHIs), soil, weather conditions and plot size not stated, harvest Sept; sampling at random, sample size 2 kg; storage at –20°C (storage time not stated).

[7] DI-1304, De Wilde, 1978c, non-GLP, harvest Aug/Sept, equipment, soil, weather conditions, plot size and sampling method not stated, 2 kg fruits; storage at –20°C (storage time not stated).

[8] DI-9620, Nowacki, 1995, non-GLP. Plot size: 6x12 trees (0.15 ha), field samples about 4 kg. Samples were harvested as immature fruits. Storage at –20°C (storage time not stated). Samples are mean of three or four replicate analytical portions. Equipment, soil, weather conditions and sampling method not stated.

[8a] DI-4309, Buisman, 1983, non-GLP. Equipment, soil type, weather conditions, plot size and sample size not stated, harvest period Aug/Sept, sampling at random, storage at –20 °C (storage time not stated). Magre: combination treatment with 0.0075 kg ai/hl Dimilin WP 5% plus 0.022 kg ai/hl Tumar EC 22.08%.

[9] DI-6155, Buisman and Snijders, 1987c, non-GLP. Decline trial (same last treatment date, different harvest times), tractor mounted sprayer with hand gun, spray volume 2500 l/ha, harvest Aug/Sept, storage at –20°C (storage time not stated). Soil, weather conditions, plot size and sampling not stated.

[10] DI-7240, Pouwelse, 1989, non-GLP. Decline trial (different treatment dates, same harvest date), spray to run off using a high pressure handgun (knapsack), harvest Sept, storage at –20°C for 1.5 months. Soil, weather conditions, plot size and sampling not stated.

[11] DI-901, De Wilde, 1977c, non-GLP. Decline trials (same last treatment time, different harvest times), harvest Jan/Febr/Mar. Equipment, soil, weather conditions, plot size sampling and storage conditions not stated.

[12] DI-914, Goto, 1977, non-GLP, mainly decline trials (same treatment date, different harvest times), harvest Sept/Oct, spray volume 5000-7000 l/ha, equipment, soil, weather conditions, plot size and sampling not stated, samples are assumed to be duplicate field samples (not stated), storage at –20°C for 192-220 days.

[13] DI-4842, De Wilde and Buisman, 1985a, non-GLP. Replicates were replicate field samples, harvest Aug/Sept/Oct, storage at –20°C (storage time not stated). In Nova Scotia the spray volume was 3300 l/ha. Equipment, soil, weather conditions, plot size and sampling not stated.

[14] DI-2272, De Wilde, 1977g, non-GLP, decay trials (same last treatment date, different harvest times). Equipment, soil type, weather conditions and plot size not stated. Harvest period Aug/Sept/Oct, sample size 2.4-8.6 kg, sampling method not stated, storage at –20 °C (storage time not stated).

[15] DI-1253, Buisman *et al.*, 1976c, non-GLP, equipment, soil not stated, harvest Aug/Sept/Oct, sampling at random, storage at –20°C (storage time not stated). French and Spanish trials: plot size, sample size and weather conditions not stated, UK trials: very hot and dry in August, wet in September, sampling 48-64 fruits at random from 10-32 trees.

[16] DI-2259, De Wilde, 1977e, non-GLP, decline trials (same last treatment date, different harvest times), spray volume 2000 l/ha, equipment not stated, soil: sandy loam (Bonnigheim, Impekoven), loamy sand (Asperg), 20 trees/plot, plot size 2500 m^2, climate dry and extremely hot. Harvest Aug/Sept/Oct, 30-50 apples/sample, replicates are assumed to be field samples (not stated), storage at –25°C (storage time not stated).

[17] DI-2725, Buisman *et al.*, 1976f, non-GLP, equipment, soil, weather conditions, plot size and sampling not stated, harvest Sept, storage at –20°C (storage time not stated).

[18] DI-2723, Buisman *et al.*, 1976e, non-GLP, equipment, soil, weather conditions, plot size and sampling not stated, harvest Aug/Sept, storage at –20°C (storage time not stated). Results from Spain were disregarded because no treatment or harvest dates or intervals were reported.

[19] DI-2273, De Wilde, 1977h, non-GLP, equipment, soil, weather conditions, plot size and sampling not stated, harvest Sept, storage at –20°C (storage time not stated). Samples from Firenze (50 days PHI) are assumed to be from two replicate field plots with two field samples per plot (not stated).

[20] DI-4845, De Wilde and Buisman, 1985b, non-GLP. Replicates were replicate field samples, harvest Aug/Sept, sampling at random: 5 apples/sample, storage at –20°C (storage time not stated). Equipment, soil, plot size and weather conditions not stated.

[21] DI-779, De Wilde, 1982, non-GLP, mixed decline trials (same last treatment times with different harvest times and different last treatment times with same harvest time, indicated by treatment intervals). Equipment, soil, weather conditions and plot size not stated, harvest time Aug-Oct, sampling at random, field sample 50 fruits, storage at –20°C (storage time not stated).

[22] DI-2260, De Wilde, 1977f, non-GLP, equipment, soil and weather conditions not stated, harvest Sept, 15 trees/plot, sampling not stated, storage at –20°C (storage time not stated).

[23] DI-4693, ABC, 1984, non-GLP. Spray volume 934 or 3738 l/ha, storage frozen for 253-316 days (temperature not stated). Equipment, soil, weather conditions, plot size and sampling not stated.

[24] DI-5054, Duphar, 1985a, non-GLP. Spray volume 1402, 2336 or 3738 l/ha, cold storage (1° C) for 3 months followed by frozen storage (temperature and time not stated). Equipment, soil, weather conditions, plot size and sampling not stated.

[25] DI-6125, Ball, 1987, non-GLP. Spray volume 935 or 3738 l/ha. Replicates are assumed to be replicate field samples (not stated clearly). Equipment, soil, weather conditions, plot size, sampling and storage conditions not stated.

[26] DI-4846, Buisman and Verhaar, 1985a, non-GLP. Spray volume 630 l/ha, other treatments difolotan, captan, polyram, phosalone and plictran, sampling at random, storage at –20°C (storage time not stated). Equipment, soil, weather conditions, plot size and sample size not stated.

[27] DI-9320, Thus and Allan, 1995, 1996 (GLP). Conventional high volume spray (1500 l/ha) or mist blower low volume spray (500 l/ha). No unusual weather conditions were reported. Sample size 1.1-2.7 kg/sample, storage at –10°C for 180-262 days. Tonisvorst: sandy loam (sL), pH 6.7, 1.9% om, plot size 45.5 m^2; 10 trees 7 yr old. Kirchheim: silty loam (uL), plot size 80.5 m^2; 15 trees 5 yr old. Orsingen-Nenzingen: sandy loam (sL), plot size 39.66 m^2, 8 trees 16 yr old. Ersdorf: sandy loam (sL), plot size 35.2 m^2, 10 trees 3 yr old.

[28] DI-4292, De Wilde, 1984, non-GLP. Equipment, soil, weather conditions and sampling not stated, harvest Sept, storage at –20°C (storage time not stated). Impekhoven: plot area 200 m^2, spray volume 1600 l/ha; Hochstadt: plot size 25 trees, spray volume 500 l/ha.

[29] DI-634, De Wilde, 1979b, non-GLP, decline trials (same last treatment time, different harvest times), spray volume 500-750 l/ha water, plot size 5000 m^2 (Impekhoven) and 288 m^2 (Stockach), 4-8 year old apple trees, in Stockach other pesticides were used during the treatment time (Nimrod, Vinicoll, Orthocid), harvest Aug/Sept, sampling at random, field sample 50 fruits, laboratory sample 700-800 g; storage at –20°C (storage time not stated).

[30] DI-129, Buisman, 1980, non-GLP; harvest time Sept/Oct 1979. Sampling at random. Equipment, soil, weather conditions, plot size, sample size and storage conditions not stated.

Table 119. Residues of diflubenzuron and CPU in whole pears.

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	Ref
single applications								
Netherlands, Gronsveld, 1975, Conference/ Doyenne d. C.	WP 250	ns	1x0.010	-	83	<0.03	na	[5]
Netherlands, Zevenbergse Hoek, 1976, Conference/ Doyenne d. C.	WP 250	ns	1x0.020	-	104	0.022	na	[6]
Netherlands, Zevenbergse Hoek, 1976, Conference/ Doyenne d. C.	SC 400	ns	1x0.020	-	104	0.048	na	[6]
Netherlands, Zevenbergse Hoek, 1976, Conference/ Doyenne d. C.	WP 250	ns	1x0.020*	-	104	0.094	na	[6]
Netherlands, Kraggenburg, 1976, Beurre Hardy	WP 250	ns	1x0.020	-	91	0.052	na	[6]
Netherlands, Gronsveld, 1975, Conference/ Doyenne d. C.	WP 250	ns	1x0.020	-	83	0.048	na	[5]
Netherlands, Kraggenburg, 1976, Beurre Hardy	WP 250	ns	1x0.020*	-	42	0.090	na	[6]
Netherlands, Zevenbergse Hoek, 1976, Conference/ Doyenne d. C.	WP 250	ns	1x0.030	-	104	0.034	na	[6]
Netherlands, Kraggenburg, 1976, Beurre Hardy	WP 250	ns	1x0.030	-	91	0.038	na	[6]
Netherlands, Zevenbergse Hoek, 1976, Conference/ Doyenne d. C.	WP 250	ns	1x0.040	-	104	0.080	na	[6]
Netherlands, Kraggenburg, 1976, Beurre Hardy	WP 250	ns	1x0.040	-	91	0.078	na	[6]
Netherlands, Wemeldinge, 1975, Conference	WP 250	ns	1x0.040	-	86	0.056	na	[5]
Netherlands, Gronsveld, 1975, Conference/ Doyenne d. C.	WP 250	ns	1x0.040	-	83	0.072	na	[5]
Netherlands, Krabbendijke, 1975, Conference	WP 250	ns	1x0.040	-	65	0.24	na	[5]
U.K., E. Farleigh, Kent; 1982, Conference	WP 250	1x0.15	1x0.015	-	21 28 35	0.062 0.066 0.098	na	[7]
U.K., E. Farleigh, Kent; 1982, Comice	WP 250	1x0.15	1x0.015	-	14 21 28	0.11 0.056 0.085	na	[7]
U.K., E. Farleigh, Kent; 1982, Conference	WP 250	1x0.15	1x0.015[1]	-	21 28 35	0.13 0.064 0.13	na	[7]
U.K., E. Farleigh, Kent; 1982, Comice	WP 250	1x0.15	1x0.015[1]	-	14 21 28	0.073 0.083 0.061	na	[7]

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	Ref
Italy, Cuneo, 1982, Madernassa	WP 050	ns	1x0.0075	-	15	0.10	na	8
Italy, Frangarto, BZ, 1985, Kaiser Alexander	WP 050	1x0.31	1x0.012	-	0	0.55	na	9
					13	0.33		
					19	0.32		
					26	0.28		
					33	0.29		
					42	0.17		
S. Africa, Houwhoek, Western Cape, 1977	WP 250	ns	1x0.020	-	1	0.44	na	10
					2	0.17		
					4	0.30		
					8	0.13		
					16	0.22		
					32	0.33		
S. Africa, Elgin, Western Cape, 1977	WP 250	ns	1x0.020	-	1	0.24	na	10
					2	0.43		
					4	0.21		
					8	0.27		
					16	0.11		
					32	0.21		
S. Africa, Houwhoek, Western Cape, 1977	WP 250	ns	1x0.040	-	1	1.2	na	10
					2	1.4		
					4	0.95		
					8	1.1		
					16	0.91		
					32	1.1		
S. Africa, Elgin, Western Cape, 1977	WP 250	ns	1x0.040	-	1	1.7	na	10
					2	2.2		
					4	1.3		
					8	1.2		
					16	1.0		
					32	0.61		
USA, (Parlier, CA), 1997, Shinsui	WP 250	1x0.84	1x0.034[2]	-	119	<0.05 (2); mean <0.05	<0.001 (2); mean <0.001	11
USA, (Hood River, OR), 1997, Bosc	WP 250	1x0.84	1x0.041[2]	-	184	<0.05 (2); mean <0.05	<0.001 (2); mean <0.001	11
USA, (Wenatchee, WA), 1997, Bartlett	WP 250	1x0.84	1x0.058[2]	-	141	<0.05 (2); mean <0.05	<0.001 (2); mean <0.001	11
USA, (Wenatchee, WA), 1997, De Anjou	WP 250	1x0.84	1x0.059[2]	-	141	<0.05 (2); mean <0.05	<0.001 (2); mean <0.001	11
USA, (Alton, NY), 1997, Bartlett	WP 250	1x0.84	1x0.090[2]	-	112	<0.05 (2); mean <0.05	<0.001 (2); mean <0.001	11
Canada, (Vineland Station, ON), 1997, Bartlett	WP 250	1x0.84	1x0.090[2]	-	113	<0.05 (2); mean <0.05	<0.001 (2); mean <0.001	11
two applications								
Netherlands, Krabbendijke, 1976, Bonne Louise d'Avranches	WP 250	ns	2x0.010	45	47	0.13	na	6
Netherlands, 's-Graveland, 1976, Triumphe de Vienne	WP 250	ns	2x0.010	24	91	0.024	na	6
Netherlands, Kraggenburg, 1976, Beurre Hardy	SC 400[3]; WP 250[4]	ns	1x0.020* 1x0.010	69	22	0.12	na	6
Netherlands, Krabbendijke, 1976, Bonne Louise d'Avranches	WP 250	ns	2x0.020	45	47	0.15	na	6
Netherlands, 's-Graveland, 1976, Triumphe de Vienne	WP 250	ns	2x0.020	24	91	0.058	na	6
Netherlands, Krabbendijke, 1975, Conference	WP 250	ns	1x0.020; 1x0.040	43	65	0.20	na	5
Netherlands, Krabbendijke, 1976, Bonne Louise d'Avranches	WP 250	ns	2x0.040	45	47	0.31	na	6
Netherlands, Krabbendijke, 1975, Conference	WP 250	ns	2x0.040	43	65	0.27	na	5

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	Ref
Netherlands, ’s-Graveland, 1976, Triumphe de Vienne	WP 250	ns	2x0.040	24	91	0.084	na	6
USA, (Upper Lake, CA), 1996, Bartlett	WP 250	0.84; 0.28	1x0.18; 1x0.060	20	63	0.096, 0.12 (2); mean 0.11	0.001 (3); mean 0.001	12
USA, (Zillah, WA), 1996, Bartlett	WP 250	0.84; 0.28	1x0.18; 1x0.060	21	63	0.054, 0.060, 0.071; mean 0.062	0.002 (2), 0.004; mean 0.003	12
three applications								
Netherlands, Wemeldinge, 1975, Conference	WP 250	ns	2x0.020; 1x0.040	17, 22	86	0.17	na	5
Netherlands, Wemeldinge, 1975, Conference	WP 250	ns	3x0.040	17, 22	86	0.20	na	5
S. France, Cabannes (13), 1979; Alexandrine Douillard	WP 250	3x0.10	3x0.010	58, 26	16	0.12	na	13
S. France, Estillac (47), 1979; Williams	WP 250	3x0.10	3x0.010	14, 15	33	0.40	na	13
S. France, Estillac (47), 1979; Williams	WP 250	3x0.10	3x0.020	24, 23	15	0.30	na	13
S. Africa, Groot Drakenstein, Western Cape, 1976/77, -	WP 250	ns	3x0.030	33, 32	31	0.22	na	10
USA, Ryde, CA, 1983	WP 250	3x0.21	3x0.006	30, 36	28	0.14	na	14
USA, Wapato, WA, 1983	WP 250	3x0.21	3x0.006	29, 35	27	0.29	na	14
USA, Ryde, CA, 1983	WP 250	3x0.21	3x0.022	30, 36	28	0.14	na	14
USA, Hood River, OR, 1983	WP 250	3x0.21	3x0.022	33, 30	28	0.22	na	14
USA, Placerville, CA, 1984, Bartlett	WP 250	3x0.28	3x0.0075	29, 31	28	0.19	na	15
USA, Wenatchee, WA, 1984, Bartlett	WP 250	3x0.28	3x0.0075	21, 53	28	0.13	na	15
USA, Bennington, VT, 1986, Bosc	WP 250	3x0.28	3x0.0075	14, 111	14 28	0.46 0.32;	na	16
USA, Bennington, VT, 1986, Anjou	WP 250	3x0.28	3x0.0075	14, 111	28	0.37	na	16
USA, Westford, MA, 1986, Bosc	WP 250	3x0.28	3x0.0075	26, 95	14 28	0.44 0.38	na	16
USA, Highland, NY, 1986, Bosc	WP 250	3x0.28	3x0.0075	14, 78	14 28	0.22 0.15	na	16
USA, Hood River, OR, 1984, D’Anjou	WP 250	3x0.28	3x0.012	22, 36	29	0.18	na	15
USA, Zillah, WA, 1984, Bartlett	WP 250	3x0.28	3x0.020	29, 47	28	0.32	na	15
USA, Fenneville, MI, 1986, Bartlett	WP 250	3x0.28	3x0.022	30, 23	14	0.19	na	16
USA, Ryde, CA, 1983, -	WP 250	3x0.42	3x0.011	30, 36	28	0.38	na	14
USA, Wapato, WA, 1983, -	WP 250	3x0.42	3x0.011	29, 35	27	0.53	na	14
USA, Ryde, CA, 1983, -	WP 250	3x0.42	3x0.045	30, 36	28	0.23	na	14
USA, Hood River, OR, 1983, -	WP 250	3x0.42	3x0.045	33, 30	28	0.54	na	14
USA, Wolcott, NY, 1986, Bartlett	WP 250	3x0.84	3x0.022	12, 80	28	1.5; 1.6; mean 1.6	na	16
four applications								
N. France, Bazouges s. Loire (72), 1979; Doyenne d. C.	WP 250	4x0.1	4x0.010	26, 16, 26	17	0.14	na	13
USA, Hood, CA, 1984, Bartlett	WP 250	4x0.28	4x0.0075	29, 31, 24	14	0.25	na	15

Country, location, year, variety	Form	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	Ref
USA, Wenatchee, WA, 1984, Bartlett	WP 250	4x0.28	4x0.0075	21, 53, 14	14	0.23	na	15
USA, Hood River, OR, 1984, D'Anjou	WP 250	4x0.28	4x0.012	22, 35, 15	14	0.35	na	15
USA, (Parlier, CA), 1997, Shinsui	WP 250	4x0.28	3x0.012; 1x0.013	21, 21, 21	14	0.10, 0.17; mean 0.14; 0.14, 0.18; mean 0.16	0.004 (2); mean 0.004; <0.001, 0.002; mean 0.002	11
USA, Wenatchee, (WA), 1997, De Anjou	WP 250	4x0.28	1x0.019; 1x0.012 1x0.014; 1x0.013	19, 23, 20	15	0.18, 0.20; mean 0.19	0.003, 0.004; mean 0.004	11
USA, Wenatchee, (WA), 1997, Bartlett	WP 250	4x0.28	1x0.018; 1x0.012; 1x0.014; 1x0.013	19, 23, 20	15	0.27, 0.32; mean 0.29	0.005, 0.008; mean 0.006	11
USA, Hood River, (OR), 1997, Bosc	WP 250	4x0.28	1x0.017; 3x0.014	21, 21, 21	13	0.32, 0.35; mean 0.34	0.004, 0.006; mean 0.005	11
USA, Zillah, (WA), 1984, Bartlett	WP 250	4x0.28	4x0.020	29, 47, 14	14	0.75	na	15
USA, Alton, (NY), 1997, Bartlett	WP 250	4x0.28	4x0.030	21, 22, 20	15	0.22, 0.23; mean 0.23	0.016, 0.025; mean 0.020	11
Canada, Vineland Station, ON), 1997, Bartlett	WP 250	4x0.28	4x0.030	21, 21, 20	14	1.4 (2); mean 1.4	<0.001 (2); mean <0.001	11
USA, Upper Lake (CA), 1996, Bartlett	WP 250	4x0.28	4x0.060	20, 28, 21	14	0.18, 0.21, 0.32; mean 0.24	0.001 (2); 0.002; mean 0.001	12
USA, Zillah, (WA), 1996, Bartlett	WP 250	4x0.28	4x0.060	21, 28, 22	14	0.097, 0.24, 0.25; mean 0.20	0.002, 0.004, 0.005; mean 0.004	12
five fold applications								
S. France, Moissac, 1976, Alex. Douillard/Beurre Hardy	WP 250	ns	2x0.030; 3x0.025	22, 21, 19, 20	44	0.26	na	17

* plus 0.3% crop oil

[1] plus 3 l/ha sunspray oil

[2] including 18.7-56 l/ha dormant oil

[3] formulation for first treatment

[4] formulation for second treatment

[5] DI-2286, De Wilde, 1977b, non-GLP. sampling at random, 100 fruits, storage at –20°C (time not stated). Equipment, soil, weather conditions and plot size not stated.

[6] DI-1314, De Wilde, 1977a, non-GLP, sampling at random, 50-100 fruits, 7-20 kg/sample. Equipment, soil, weather conditions, plot size and storage conditions not stated. Trials with experimental formulations (PH 60-41 and PH 60-42) were disregarded.

[7] DI-4381, Buisman, 1984, non-GLP. Decline trials (different last treatment times, same harvest time), spray volume 1000 l/ha, motorised knapsack mist blower, sampling at random, 2 kg/sample, storage at –20°C (time not stated). Soil, weather conditions and plot size not stated.

[8] DI-4309, Buisman, 1983, non-GLP. Equipment, soil, weather conditions, plot size and sample size not stated, harvest Aug/Sept, sampling at random, storage at –20°C (storage time not stated). Magre: combination treatment with 0.0075 kg ai/hl Dimilin WP 5% plus 0.022 kg ai/hl Tumar EC 22.08%.

[9] DI-6142, Buisman and Snijders, 1987a, GLP. Spray volume 2500 l/ha, tractor-mounted sprayer with hand gun, storage at –20°C (time not stated). Soil, weather conditions, plot size and sampling not stated.

[10] DI-901, De Wilde, 1977c, non-GLP. Decline trials (same last treatment time, different harvest times), harvest Jan/Febr/Mar. Equipment, soil, weather conditions, plot size sampling and storage conditions not stated.

[11] IR-4, Dorschner and Gaydosh 2000, GLP. Spray volume 935-2500 l/ha, tractor-, truck- or trailer-mounted air blast sprayers, no unusual weather conditions (Mar-Oct, temp -3-30°C; total rain 0-18 cm), USDA soil type: loam (Alton, Hood River), loamy sand (Wenatchee), sandy loam (Parlier, Vineland Station). Fruit selection at random from high and low sections, inside and outside trees, plot size 85-223 m^2; 2 field samples/plot; 24 fruits; 2.7-8.2 kg/sample. Storage frozen for 208-280 days for diflubenzuron, 159-286 days for CPU and 138-203 days for PCA (temperature not stated).

[12] RP-96025, Gaydosh and Rose, 1999, GLP. Spray volume 467 l/ha, tractor-mounted air blast sprayers, no unusual weather conditions (May-Aug, temp 2-43°C; total rain 0-1 cm), USDA soil type: loam (Upper Lake, pH 7.4, 4.8% om, CEC 25.2; Zillah, pH 8.0, 1.2% om, CEC 20.8). Fruit selection at random from upper, lower and middle sections of the trees, 12-18

trees/plot; plot size 214-446 m^2; 3 field samples/plot; 24 fruits; 3.2 kg/sample. Storage frozen for 68-75 days for diflubenzuron, 175-178 days for CPU, 292-298 days for PCA (temperature not stated).

[13] DI-875, Buisman, 1981, non-GLP. Spray volume 1000 l/ha, sampling at random, storage at –20°C (time not stated). Equipment, soil, weather conditions, plot size and sample size not stated. Samples from Bazouges sur Loire, were analysed in triplicate; others were single.

[14] DI-4693, ABC, 1984, non-GLP. Spray volume 934 or 3738 l/ha, storage frozen for 253-316 days (temperature not stated). Equipment, soil, weather conditions, plot size and sampling not stated.

[15] DI-5054, Duphar, 1985a, non-GLP. Spray volume 1402, 2336 or 3738 l/ha, cold storage (1° C) for 3 months followed by frozen storage (temperature and time not stated). Equipment, soil, weather conditions, plot size and sampling not stated.

[16] DI-6125, Ball, 1987, non-GLP. Spray volume 935 or 3738 l/ha. Replicates are assumed to be replicate field samples (not stated clearly). Equipment, soil, weather conditions, plot size, sampling and storage conditions not stated.

[17] DI-2022, De Wilde, 1977d, non-GLP. Fruits from trees mixed with fallen fruits. Equipment, soil, weather conditions, plot size, sample size and storage conditions not stated.

Stone fruits (group 003)

Results of trials on peaches are shown in Table 120 and on plums in Table 121. The samples were analysed by GC-ECD method LAI 3-86-6 for diflubenzuron, GC-ECD method LAI 3-86-9 for CPU (1997 trials), HPLC-MS method H for CPU (1998 trials) and method PTRL 645W for PCA. From the reports it is not clear whether samples were analysed with or without stones.

In trials in the USA in 1997 (Gaydosh 1999b, GLP) and 1998 (Gaydosh 2000a, GLP) on peaches residues were corrected for concurrent method recoveries if <100% (63%-101% for diflubenzuron, 62%-128% for CPU, 61%-103% for PCA), but not for matrix interferences (<0.005 mg/kg diflubenzuron, max 0.034 mg/kg CPU (1997 trials) and <0.01 mg/kg CPU (1998 trials), <0.005 mg/kg PCA) (Table 74 for diflubenzuron, Tables 73 and 83 for CPU, and Table 93 for PCA). Results for PCA were <0.005 mg/kg for all samples and are not tabulated. Storage times for peaches (76-407 days for CPU and 34-285 days for PCA) were much longer than the stability periods of 3 months for CPU and 1-2 weeks for PCA, so the CPU and PCA results are not considered valid.

Table 120. Residues of diflubenzuron and CPU[1] in unwashed peaches in the USA.

Location, year, variety	Form.	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	Ref.
two applications								
(Sultana, CA), 1997, Diamond Princess	WP 250	1x0.56; 1x0.28	1x0.071 1x0.030	20	97	0.009 (3); mean 0.009	<0.005 (3); mean <0.005	[2]
(Ripon, CA), 1997, Starns	WP 250	1x0.56; 1x0.28	1x0.12 1x0.060	27	148	0.010 (2), 0.012; mean 0.011	<0.005 (3); mean <0.005	[2]
(Knightdale, NC), 1997, Candor	WP 250	1x0.56; 1x0.28	1x0.073 1x0.037	25	78	0.019, 0.020, 0.022; mean 0.020	<0.005 (3); mean <0.005	[2]
(Barto, PA), 1998, Redhaven	WP 250	1x0.56; 1x0.28	1x0.058 1x0.062	22	96	<0.05 (2); mean <0.05	<0.01 (2); mean <0.01	[3]
(Conklin, MI), 1998, Bellaire	WP 250	1x0.56; 1x0.28	2x0.060	30	93	<0.05 (2); mean <0.05	<0.01 (2); mean <0.01	[3]
(Escalon, CA), 1998, -	WP 250	1x0.56; 1x0.28	1x0.058 1x0.060	40	158	<0.05 (2); mean <0.05	<0.01 (2); mean <0.01	[3]
(Cochran, GA), 1998, Harvester	WP 250	1x0.56; 1x0.28	1x0.058 1x0.062	19	94	<0.05 (2); mean <0.05	<0.01 (2); mean <0.01	[3]
(Mustang, OK), 1998, Redhaven	WP 250	1x0.56; 1x0.28	2x0.062	40	97	<0.05 (2); mean <0.05	<0.01 (2); mean <0.01	[3]
(Monetta, SC), 1998, Contender	WP 250	1x0.56; 1x0.28	1x0.059 1x0.061	39	93	<0.05 (2); mean <0.05	<0.01 (2); mean <0.01	[3]
(Escalon, CA), 1998, -	SC 240	1x0.56; 1x0.28	1x0.059 1x0.061	40	158	<0.05 (2); mean <0.05	<0.01 (2); mean <0.01	[3]
(Cochran, GA), 1998, Harvester	SC 240	1x0.56; 1x0.28	2x0.060	19	94	<0.05 (2); mean <0.05	<0.01 (2); mean <0.01	[3]
(Escalon, CA), 1998, -	WG 800	1x0.56; 1x0.28	2x0.060	40	158	<0.05 (2); mean <0.05	<0.01 (2); mean <0.01	[3]
(Cochran, GA), 1998, Harvester	WG 800	1x0.56; 1x0.28	2x0.060	19	94	<0.05 (2); mean <0.05	<0.01 (2); mean <0.01	[3]

Location, year, variety	Form.	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	Ref.
three applications								
(Sultana, CA), 1997, Diamond Princess	WP 250	1x0.58; 2x0.27	1x0.074 2x0.029	27, 69	14	0.59, 0.60, 0.66; mean 0.62;	0.006 (2), 0.007; mean 0.006;	[2]
					28	0.28 (3); mean 0.28	<0.005 (3); mean <0.005	
(Ripon, CA), 1997, Starns	WP 250	1x0.56; 1x0.29 1x0.28	1x0.12 1x0.061 1x0.030	27, 134	14	0.15, 0.17, 0.20; mean 0.17;	<0.005 (3); mean <0.005;	[2]
					28	0.14, 0.15, 0.18; mean 0.16	<0.005 (3); mean <0.005	
(Knightdale, NC), 1997, Candor	WP 250	1x0.56; 2x0.28	1x0.074 2x0.037	32, 64	14	0.21, 0.22 (2); mean 0.22;	<0.005 (3); mean <0.005;	[2]
					28	0.16 (3); mean 0.16	<0.005 (2), 0.019; mean 0.010	

[1] PCA was also determined but was <0.005 mg/kg in all samples

[2] RP-97008, Gaydosh 1999b, GLP, 2-3 applications: the first during dormancy, just before bud swell, the second at petal fall and the third just before harvest. Spray volume 467-935 l/ha, including 4.7-75 l/ha dormant oil, for the first application. In Starns the wrong spray volume was used. Tractor-mounted air blast sprayers. No unusual weather conditions (Feb-Aug, temp -2-38°C; total rain 1-38 cm), except at Knightdale (NC) where very hot weather occurred before collecting the DAT=28 samples, resulting in most fruits being overripe. USDA soil type: loam (Sultana), loamy sand (Ripon, Knightdale). Harvest Jun/Aug. Fruit selection at random from the top, centre and bottom sections of the trees, inside and outside trees, >12 trees/plot; plot size 388-595 m^2; 3 field samples/plot; 36 fruits/sample; 3.2-5.9 kg/sample. Storage frozen for 68-293 days for diflubenzuron, 76-402 days for CPU and 279-336 days for PCA (temperature not stated).

[3].RP-98001, Gaydosh 2000a, GLP, 2 applications: the first during dormancy, just before bud swell and the second at petal fall. Spray volume 467-935 l/ha, including 18.7 l/ha dormant oil for the first application. Tractor-mounted air blast sprayers. No unusual weather conditions (Feb-Sept, temp -8-41°C; total rain 21-52 cm), except at Conklin (MI) where winds of 120 mph at DAT=27 and at Cochran (GA) where rain and temperature may have affected the yield. USDA soil type: gravely silt loam (Barto), loam (Conklin), sandy loam (Escalon, Mustang), loamy sand (Cochran), sand (Monetta). Harvest Jul/Aug/Sept. Fruit selection from all quadrants from inner trees and/or inner canopies of outer trees, 12-16 trees per plot; plot size 223-595 m^2; 2 field samples/plot; 36 fruits/sample; >3 kg/sample. Storage frozen for 35-204 days for diflubenzuron, 323-407 days for CPU, 34-285 days for PCA (temperature not stated).

In the trials on plums in the USA in 1998 (Gaydosh 2000b, GLP) residues were corrected for concurrent method recoveries if <100% (69%-91% for diflubenzuron, 67%-138% for CPU and 90%-111% for PCA), but not for matrix interferences (<0.05 mg/kg diflubenzuron, <0.005 mg/kg CPU, <0.005 mg/kg PCA) (Table 74 for diflubenzuron, Table 73 for CPU and Table 93 for PCA). CPU and PCA were below 0.005 mg/kg in all the samples and are not tabulated. Because the storage times (266-326 days for CPU and 231-298 days for PCA) were much longer than the stability periods for CPU and PCA, the CPU and PCA results are not considered valid.

Table 121. Diflubenzuron concentrations in unwashed plums, USA, 1998. [1]

Location, year, variety	Form.	kg ai/ha	kg ai/hl	Interval (days)	PHI, days	diflubenzuron (mg/kg)
Conklin, (MI), Stanley	WP 250	1x0.56 1x0.28	2x0.060	34	104	<0.05 (2); mean <0.05
Madera, (CA), Autumn Butte	WP 250	1x0.57 1x0.29	2x0.060	28	196	<0.05 (2); mean <0.05
Sultana, (CA), Angelos	WP 250	1x0.57 1x0.27	1x0.061 1x0.060	22	152	<0.05 (2); mean <0.05
Forest Grove, (OR), Italian prunes	WP 250	1x0.56 1x0.28	2x0.060	43	134	<0.05 (2); mean <0.05
Ivanhoe, (CA), Simka	WP 250	1x0.56 1x0.28	1x0.059 1x0.061	49	112	<0.05 (2); mean <0.05
Forest Grove, (OR), Italian prunes	SC 240	1x0.55 1x0.28	1x0.059 1x0.060	43	134	<0.05 (2); mean <0.05
Ivanhoe, (CA), Simka	SC 240	1x0.56 1x0.28	2x0.059	49	112	<0.05 (2); mean <0.05
Forest Grove, (OR), Italian prunes	WG 800	1x0.56 1x0.28	2x0.060	43	134	<0.05 (2); mean <0.05
Ivanhoe, (CA),	WG	1x0.56	1x0.059	49	112	<0.05 (2); mean <0.05

Location, year, variety	Form.	kg ai/ha	kg ai/hl	Interval (days)	PHI, days	diflubenzuron (mg/kg)
Simka	800	1x0.28	1x0.060			
Woodland, (CA), French prunes	WP 250	1x2.2 1x1.1	2x0.24	26	147	0.15, 0.21; mean 0.18

[1] RP-98002, Gaydosh 2000b. GLP 2 applications: the first during dormancy, just before bud swell, the second at petal fall. At Woodland application was at a fourfold rate. Spray volume 467-935 l/ha, including 18.7 l/ha dormant oil for the first application. Air blast sprayers or a back pack mist blower (Woodland). No unusual weather conditions (Feb-Sept, temp -2-45°C; total rain 22-28 cm). USDA soil type: loam (Conklin, Sultana), sandy loam (Madera, Ivanhoe), silt loam (Forest Grove), clay (Woodland). Harvest Jul/Aug/Sept. Fruit selection at random from all quadrants of inner trees or inner canopies of outer trees, 12-16 trees/plot; plot size 321-482 m^2; 2 field samples/plot, 3.2-6.8 kg/sample. Storage frozen for 127-200 days for diflubenzuron, 266-326 days for CPU, 231-298 days for PCA (temperature not stated).

Berries and other small fruits (group 004)

Results of trials on blackcurrants and gooseberries in the UK in 1978 (De Wilde, 1980, non-GLP) are shown in Table 122.

Samples were analysed for diflubenzuron by method LAI 3-86-2. Residues were not corrected for concurrent method recoveries (70%-102% for blackcurrants and 88%-97% for gooseberries) or matrix interferences (< 0.003 mg/kg diflubenzuron = 0.3xLOQ, Table 48).

Table 122. Residues of diflubenzuron in unwashed blackcurrants and gooseberries. [1]

Location, year, variety	Form.	kg ai/ha	kg ai/hl	PHI, days	diflubenzuron (mg/kg)
Blackcurrants					
UK, Rolvenden Layne, 1978, Wellington	WP 250	1x0.25	ns	13 20 27	0.030 0.038 0.021
Gooseberries					
UK, Shatterling, Kent, 1978, Careless	WP 250	1x0.25	ns	13	0.35

[1] DI-201, De Wilde, 1980, non-GLP, harvest July/Aug, storage at –20°C (time not stated). Equipment, soil, weather conditions, plot size and sampling not stated.

Brassica vegetables (group 010)

Results of trials on head cabbage and Brussels sprouts are shown in Table 123.

The trials in The Netherlands in 1975 (De Wilde, 1977i) on cauliflower were excluded because dosage rates were expressed as g/plant.

In non-GLP trials in The Netherlands in 1974 and Germany in 1975 (Buisman *et al.*, 1976g) on head cabbages samples were analysed for diflubenzuron by HPLC method LAI 3-86-2. Residues were not corrected for concurrent method recoveries (64%-110%) or matrix interferences (max. 0.47 mg/kg diflubenzuron, Table 48). Because of high matrix interferences, results could not be evaluated.

Samples from non-GLP trials on head cabbages in the UK in 1978 (De Wilde, 1980) were analysed for diflubenzuron by HPLC method LAI 3-86-3. Residues were not corrected for concurrent method recoveries (63%-82%) or matrix interferences (max. 0.012 mg/kg diflubenzuron, Table 49). Because of matrix interferences, results below 0.04 mg/kg are not considered valid.

In non-GLP trials on cabbages in Brazil in 1986 (Buisman and Dijkstra, 1988) samples were analysed for diflubenzuron by HPLC method A. Residues were not corrected for concurrent method recoveries (93%-98%) or matrix interferences (<0.18 mg/kg diflubenzuron = LOQ, Table 55).

Samples from non-GLP trials on Brussels sprouts in The Netherlands in 1976 (De Wilde, 1978d) were analysed for diflubenzuron by HPLC method LAI 3-86-2. Residues were not corrected for concurrent method recoveries (66%-114%) or matrix interferences (max. 0.058 mg/kg diflubenzuron, Table 48). Because of matrix interferences, results below 0.2 mg/kg are not considered valid.

In non-GLP trials in the UK in 1977 and 1978 (De Wilde, 1978e, 1980) on Brussels sprouts samples were analysed for diflubenzuron by GC-ECD method LAI 3-86-6. Residues were not corrected for concurrent method recoveries (76%-92%) or matrix interferences (max. 0.004 mg/kg diflubenzuron, Table 74). The results from the 1977 trials could not be evaluated because of high levels in control samples (up to 0.46 mg/kg).

Table 123. Residues of diflubenzuron in brassica vegetables.

Location, year, variety	Form.	kg ai/ha	kg ai/hl	interval days	PHI, days	diflubenzuron (mg/kg)	Ref
Brussels sprouts							
Netherlands, Groessen, 1976, -	WP 250	ns	4x0.0010	18, 10, 55	16	0.069	1
Netherlands, Groessen, 1976, -	WP 250	ns	4x0.010	18, 10, 55	16	0.30	1
Netherlands, Espel, 1976, -	WP 250	ns	5x0.0010	20, 17, 13, 14	58	0.072	1
Netherlands, Espel, 1976, -	WP 250	ns	5x0.010	20, 17, 13, 14	58	0.33	1
UK, Sweffling, Suffolk, 1977, F1 hybrid	WP 250	1x0.25	ns	-	7 14 21 28	0.69 0.60 0.45 0.50	2
UK, Thornhaugh, 1978, Lunet	WP 250	1x0.15	ns	-	38	0.27	3
Head cabbages							
Netherlands, Opperdoes, 1974, Savoy cabbage	WP 250	1x0.25	1x0.010	-	0 7 14 28 42	0.21, 0.22, 0.36, 0.66 0.072, 0.12, 0.20 (2) 0.048, 0.096, 0.15, 0.19 <0.03 (4) <0.03 (4)	4
Netherlands, Opperdoes, 1974, Savoy cabbage	WP 250	1x0.25	1x0.010	-	0 7 14 28 42	<0.03, 0.13, 0.20, 0.41 0.064, 0.076, 0.092, 0.18 <0.03, 0.056, 0.080, 0.096 0.056 (2), 0.072, 0.100 <0.03 (2), 0.036, 0.048	4
Germany, Schifferstadt, 1975, oxheart cabbage	WP 250	1x0.060	1x0.010	-	38	<0.05	4
Germany, Dusseldorf-Hamm, 1975, oxheart cabbage	WP 250	1x0.10	1x0.010	-	0 7 14 21 28 35	1.5, 1.7, 2.4, 2.5 0.56, 0.74, 0.80, 1.0 0.32, 0.39 (2), 0.48 0.14, 0.17, 0.21, 0.26 0.052, 0.056, 0.096 (2) <0.05 (3), 0.052	4
UK, 1978, Firmhead	WP 250	2x0.088	ns	ns	13 29 43	0.058 0.003 0.000	3
Brazil, Porto Alegre, RS State, 1986, -	WP 250	1x0.075	ns	-	4	1.0	5
Brazil, Porto Alegre, RS State, 1986, -	WP 250	1x0.15	ns	-	4	1.5	5

ns: not stated

[1] DI-1315, De Wilde, 1978d, non-GLP, harvest October, storage at –25°C (storage time not stated). Equipment, soil, weather conditions, plot size and sampling not stated.

[2] DI-1418, De Wilde, 1978e, non-GLP, drop-leg sprayer, soil sand, 1 kg/sample, harvest Oct/Nov, storage –20°C (storage time not stated). Weather conditions and sampling method not stated.

[3] DI-201, De Wilde, 1980, non-GLP, decline trial, harvest July/Aug for cabbage and October for Brussels sprouts, soil for Brussels sprouts very fine sandy loam. Storage at –20°C (time not stated). Equipment, soil for cabbage, weather conditions, plot size and sampling not stated.

[4] DI-4334, Buisman *et al.*, 1976g, non-GLP, spray volume 600-2500 l/ha, soil loamy sand (Dusseldorf) or sandy loam (Schifferstadt); plot size: 8x31 plants; harvest July-Nov; 6 cabbage plants/field sample; sampling according to a sampling scheme, no plants taken from the outer rows. Equipment, weather conditions and sampling and storage conditions not stated.

[5] DI-6955, Buisman and Dijkstra, 1988, non-GLP. Weather warm, cloudy and humid and 80 mm rainfall during the treatment period (4 days). Harvest Nov. During shipment from Brazil to The Netherlands, samples were thawed. After arrival stored at –20°C (storage time not stated). Equipment, soil, plot size and sampling not stated.

Fruiting vegetables other than cucurbits (group 012)

Results of trials on sweet peppers, chilli peppers and tomatoes are shown in Table 124 and on mushrooms in Table 125.

Samples from trials in the USA in 1997 (Gaydosh and Puhl, 1999, GLP) on sweet and chilli peppers were analysed by GC-ECD method LAI 3-86-6 for diflubenzuron, GC-ECD method LAI-3-86-9 for CPU and method PTRL 645W for PCA. Residues were not corrected for concurrent method recoveries (82%-133% for diflubenzuron, 70%-132% for CPU, 69%-103% for PCA) or matrix interferences (<0.05 mg/kg diflubenzuron, <0.01 mg/kg CPU, <0.005 mg/kg PCA) (Table 74 for diflubenzuron, Table 83 for CPU and Table 93 for PCA). PCA levels were <0.005 mg/kg and are not tabulated. Because storage times for peppers (177-347 days) were much longer than stability period for PCA (1-2 weeks), PCA results in peppers are not considered valid.

In non-GLP trials on tomatoes in the UK in 1977 and 1978 and in Brazil in 1989 (De Wilde 1978f, 1980; Pouwelse and Van Zijtveld, 1991) samples were analysed for diflubenzuron by method LAI 3-86-2. Residues were not corrected for concurrent method recoveries (92%-112%), storage stability (74%-95% for 0.1-1.0 mg/kg in Brazilian trials) or matrix interferences (max. 0.039 mg/kg diflubenzuron, Table 48). Because of matrix interferences results below 0.2 mg/kg are not considered valid.

For non-GLP trials on tomatoes in Brazil in 1991 (Yokomizo *et al.*, 1992) field conditions were not reported. Samples were stored for 429 days (14 months) at –20°C. Because this is longer than the stability period of 12 months, the results could not be evaluated. Samples were analysed for diflubenzuron by the Brazilian HPLC method. It is not clear whether residues were corrected for concurrent method recoveries (82%-98%) or matrix interferences (<0.02 mg/kg diflubenzuron).

Table 124. Residues of diflubenzuron and CPU in peppers and diflubenzuron in tomatoes. PCA was determined in peppers, but all results were <0.005 mg/kg.

Location, year, variety	Form.	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	Ref
Sweet peppers								
USA, Lodi, (CA), 1997, Bomby	WP 250	5x0.14	5x0.050	7, 7, 7, 7	7	0.23, 0.25; mean 0.24	<0.01 (2); mean <0.01	1
USA, Noblesville, (IN), 1997, California Wonder	WP 250	5x0.14	5x0.050	7, 7, 7, 7	7	0.21, 0.26; mean 0.24	0.013, 0.014; mean 0.014	1
USA, Knightdale, (NC), 1997, Yolo Wonder	WP 250	5x0.14	5x0.050	7, 7, 7, 7	7	0.050, 0.093; mean 0.072	<0.01, 0.010; mean 0.010	1
USA, San Marcos, (CA), 1997, Jupiter	WP 250	5x0.14	5x0.050	7, 7, 7, 7	7	<0.05, 0.098; mean 0.074	<0.01, 0.038; mean 0.024	1
USA, Immokalee, (FL), 1997, California Wonder	WP 250	5x0.14	5x0.050	7, 7, 7, 7	7	<0.05, 0.12; mean 0.088	<0.01 (2); mean <0.01	1
USA, Donna, (TX), 1997, Jupiter	WP 250	5x0.14	5x0.050	7, 7, 7, 7	7	0.33 (2); mean 0.33	0.027, 0.057; mean 0.042	1
Chilli peppers								
USA, Bernard, (TX),	WP	5x0.14	5x0.050	7, 7, 7, 7	7	0.90, 0.94;	<0.01, 0.057;	1

Location, year, variety	Form.	kg ai/ha	kg ai/hl	Interval, days	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	Ref
1997, Anaheim	250					mean 0.92	mean 0.034	
USA, Rincon, (NM), 1997, Anaheim	WP 250	5x0.14	5x0.050	7, 7, 7, 7	7	0.92, 0.95; mean 0.93	<0.01 (2); mean <0.01	1
USA, San Marcos, (CA), 1997, Anaheim 23TMR	WP 250	5x0.14	5x0.050	7, 7, 7, 7	7	0.21; 0.29; mean 0.25	<0.01 (2); mean <0.01	1
Tomatoes								
UK, GCRI, 1977, -	WP 250	ns	1x0.012	-	1 3 6 7	0.40 0.24 0.13 0.075		2
UK, West of Scotland Agr. College, 1978, Money maker	WP 250	ns	1x0.015	-	1 3 5 7	1.4 1.1 0.96 0.92		3
UK, GCRI, 1978, Sonata	WP 250	1x0.25	ns	-	1 3 5 7	0.80 0.50 0.68 0.74		3
Brazil, Goianapolis, 1991, -	WP 250	1x0.062	1x0.0078	-	4	0.02[4]		4
Brazil, Sao Paulo, 1989, Rio Grande	WP 250	1x0.062	1x0.017	-	2 7 13	0.023 0.023 0.018		5
Brazil, Goianapolis, 1991, -	WP 250	1x0.12	1x0.016	-	4	0.03[4]		4
Brazil, Sao Paulo, 1989, Rio Grande	WP 250	1x0.12	1x0.034	-	2 7 13	0.036 0.069 0.066		5
Brazil, Sao Paulo, 1989, Rio Grande	WP 250	1x0.25	1x0.068	-	2 7 13	0.071 0.083 0.060		5
Brazil, Goianapolis, 1991, -	WP 250	1x0.25	1x0.031	-	4	0.035[4]		4

[1] RP-97016, Gaydosh and Puhl, 1999, GLP, 5 applications from pre-bloom or early flowering stage up to maturity stage. Spray volume 280 l/ha for each application with self propelled, back pack, bicycle mounted or tractor mounted sprayers. No unusual weather conditions (May-Jun (FL), Jun-Jul (NC, CA, TX), Jul-Aug (IN, NM), Aug-Sept (CA), Oct-Nov (TX); temp 4-37°C; total rain 0-14 cm), soil not stated. Fruit selection at random, plot size 46-237 m^2; 2 field samples/plot; 1.9-5.0 kg peppers/sample. Storage frozen for 172-340 days for diflubenzuron, 178-357 days for CPU and 177-347 days for PCA (temperature not stated)

[2] DI-4345, De Wilde, 1978f, non-GLP, 1 kg/sample. Storage at –20°C (storage time not stated). Equipment, soil, weather conditions, plot size and sampling method not stated.

[3] DI-201, De Wilde, 1980, non-GLP, decline trial (different application times, same harvest time), harvest Aug-Sept. Storage at –20°C (storage time not stated). Equipment, soil, weather conditions, plot size and sampling not stated.

[4] DI-8874, Yokomizo *et al.*, 1992, non-GLP. Spray volume 800 l/ha. Other products used: Nenhum. Harvest April, harvested as green fruits. Sample size 2 kg. Result is average of triplicate analyses (20 g each). Storage at –18±2°C for 429 days. Equipment, soil, weather conditions, plot size, sampling method not stated.

[5] DI-8333, Pouwelse and Van Zijtveld, 1991, non-GLP. Spray until run-off, spray volume 365 l/ha. Other pesticides used: mancozeb, captan, metamidophos and iprodione. Harvest Sept, 13-31 fruits/sample. Storage conditions from harvest in Brazil to arrival in The Netherlands not stated (30-50 days); thereafter storage at –20°C as chopped tomatoes for 10 months. Equipment, soil, weather conditions and sampling method not stated.

In trials in The Netherlands in 1975, 1976 and 1977 (Buisman *et al.*, 1976a; De Wilde, 1978a,b, 1979a) on mushrooms the samples were analysed for diflubenzuron by method LAI-3-86-2, CPU by GC-ECD method G and DFBA by GC-MS method LAI-3-86-10. Residues were not corrected for method recoveries (43%-128% for diflubenzuron, 23%-121% for CPU, 58%-128% for DFBA) or matrix interferences (max. 0.040 mg/kg diflubenzuron, max. 0.048 mg/kg CPU, max. 0.053 mg/kg DFBA, Table 48 for diflubenzuron, Table 82 for CPU, and Table 92 for DFBA). Because of matrix interferences and recoveries <70% or >110%, results below 0.5 mg/kg diflubenzuron, 0.2 mg/kg CPU and 0.2 mg/kg DFBA are not considered valid.

The results of trials in the UK in 1977 (De Wilde, 1977j) on mushrooms were excluded as only the results for DFBA were available.

Samples from trials in Australia in 1992 (Allan and Thus, 1996) on mushrooms were analysed by HPLC method LAI 3-86-14 for diflubenzuron, GC-ECD method LAI 3-86-9 for CPU, and GC-MS method LAI 3-86-10 for DFBA. Residues were not corrected for concurrent method recoveries (100%-102% for diflubenzuron, 85%-90% for CPU, 97%-120% for DFBA) or matrix interferences (<0.003 mg/kg diflubenzuron, <0.003 mg/kg CPU, no peaks for DFBA, Table 56 for diflubenzuron, Table 83 for CPU, and Table 92 for DFBA).

In GLP trials in the USA in 1996 and 1997 (Gaydosh 1998a, 1999c) on mushrooms samples were analysed by HPLC method LAI 3-86-13 for diflubenzuron, GC-ECD method LAI 3-86-9 for CPU, method CARDC-1248-PCA for PCA in 1996 and PTRL 645W in 1997, and method CARDC-1248-DFBA for DFBA. Residues were corrected for concurrent method recoveries if <100% (62%-112% for diflubenzuron, 68%-125% for CPU, 69%-160% for PCA, 76%-122% for DFBA), but not for matrix interferences (max 0.01 mg/kg diflubenzuron, max 0.01 mg/kg CPU, max 0.02 mg/kg PCA in 1996, and 0.0056 mg/kg in 1997, max 0.03 mg/kg DFBA, Table 53 for diflubenzuron, Table 83 for CPU, Tables 87 and 93 for PCA and Table 90 for DFBA). Because of matrix interferences results below 0.04 mg/kg diflubenzuron, 0.04 mg/kg CPU, 0.07 mg/kg PCA (1996), 0.02 mg/kg PCA (1997) and 0.1 mg/kg DFBA are not considered valid. Because storage times for mushrooms (37-146 days) are much longer than stability period for PCA (1-2 weeks), the PCA results are not considered valid.

Residues of DFBA in the 1996 US trials were consistently higher in samples from Pennsylvania than in corresponding samples from California. DFBA residues in control samples from California were <0.01-0.03 mg/kg, but those in control samples from Pennsylvania were 0.16-0.95 mg/kg. It appears that mushroom variety Amycel 2600 contains a component that interferes significantly with quantification of DFBA and these results are not considered valid.

Diflubenzuron residues in the 1996 US trials from casing applications with 0.25 kg ai/hl were higher than those with 0.068 kg ai/hl: 0.44-5.6 mg/kg and <0.01-0.14 mg/kg. At the former dilution the spray applications did not appear to completely penetrate the casing. This apparently resulted in localised high diflubenzuron concentrations, leading to higher residues in the mushrooms.

The Meeting was informed that 4 trials on mushrooms are in progress: two in the UK and two in The Netherlands. The expected completion date is December 2002.

Table 125. Residues of diflubenzuron and metabolites in fruit bodies of mushrooms.

Location, year, variety	Form	kg ai/hl	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	PCA, mg/kg	DFBA, mg/kg	Ref
Single compost treatment at 49 kg ai/ha								
USA, (Watsonville, CA), 1997, Button	WP 250	0.30	34	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005	na	3
			40	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005		
			48	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005, 0.0052; mean 0.0051		
			57	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.0092, 0.0096; mean 0.0094		
USA, (Watsonville, CA), 1997, Button	SC 480	0.30	34	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005	na	3
			40	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005		
			48	<0.01 (2);	<0.01 (2);	<0.005, 0.0053;		

Location, year, variety	Form	kg ai/hl	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	PCA, mg/kg	DFBA, mg/kg	Ref
				mean <0.01	mean <0.01	mean 0.0052		
			57	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.0078; 0.020; mean 0.014		
USA, (Tempel, PA), 1996, Amycel 2600	WP 250	0.80	37	0.02, 0.03; mean 0.02	<0.01 (2); mean <0.01	<0.01, 0.01; mean 0.01	0.20, 0.27; mean 0.24	4
			45	<0.01, 0.02; mean 0.02	<0.01 (2); mean <0.01	0.01 (2); mean 0.01	0.37, 0.42; mean 0.40	
			54	0.01 (2); mean 0.01	<0.01 (2); mean <0.01	0.01 (2); mean 0.01	0.03, 0.44; mean 0.24	
			61	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.02 (2); mean 0.02	0.80, 0.95; mean 0.88	
USA, (Tempel, PA), 1996, Amycel 2600	SC 480	0.80	19	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.27, 0.30; mean 0.28	4
			27	<0.01, 0.02; mean 0.02	<0.01 (2); mean <0.01	<0.01, 0.01; mean 0.01	1.1, 1.2; mean 1.1	
			36	<0.01, 0.01; mean 0.01	<0.01 (2); mean <0.01	0.02 (2); mean 0.02	1.8, 2.1; mean 2.0	
			43	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.02 (2); mean 0.02	1.7, 3.0; mean 2.3	
USA, (Gilroy, CA), 1996, A. bisporus	WP 250	1.2	29	<0.01, 0.01; mean 0.01	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	4
			36	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.02 (2); mean 0.02	
			46	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.01, 0.02; mean 0.02	<0.01 (2); mean <0.01	
			58	<0.01, 0.01; mean 0.01	<0.01 (2); mean <0.01	0.01 (2); mean 0.01	<0.01, 0.03; mean <0.01	
USA, (Gilroy, CA), 1996, A. bisporus	SC 480	1.2	16	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.03 (2); mean 0.03	4
			23	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.04, 0.05; mean 0.04	
			33	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.02 (2); mean 0.02	<0.01 (2); mean <0.01	
			45	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.02, 0.03; mean 0.02	0.01, 0.02; mean 0.02	
USA, (Tempel, PA), 1997, Lambert 900	WP 250	1.2	30	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005	na	3
			37	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005		
			47	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005		
			55	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.0074, 0.013; mean 0.010		
USA, (Tempel, PA), 1997, Lambert 900	SC 480	1.2	30	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005	na	3
			37	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005		
			47	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005		
			55	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005, 0.0055; mean 0.0052		
Single casing treatment at 7.5 kg ai/ha								
Australia, Rydalmere, 1992, Off-white hybrid	WP 250	0.0038	17	0.018	<0.01	na	0.38	5
			24	0.018	0.017		0.64	
			29	0.021	0.017		0.71	
Single casing treatment at 10 kg ai/ha								
The Netherlands, Beesel, 1977; Soumycel 11	WP 250	ns	21	0.042	0.013	na	0.30	6
			28	<0.01	<0.01		0.099	
			35	0.028	0.031		0.20	
			42	0.017	0.039		0.43	
			49	0.032	0.041		0.42	
The Netherlands, Beesel, 1977; Soumycel	WP 250	ns	21	0.035	0.026	na	0.60	6
			28	0.037	0.041		0.22	

Location, year, variety	Form	kg ai/hl	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	PCA, mg/kg	DFBA, mg/kg	Ref
11			35 42 49	0.062 0.019 0.014	0.065 0.036 0.029		0.57 0.29 0.44	
USA, (Watsonville, CA), 1997, Button	WP 250	0.063	19 25 33 42	<0.01 (2); mean <0.01 0.04, 0.06; mean 0.05 0.02 (2); mean 0.02 0.09 (2); mean 0.09	<0.01 (2); mean <0.01 <0.01 (2); mean <0.01 <0.01, 0.01; mean 0.01 <0.01, 0.01; mean 0.01	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005 <0.005, 0.0051; mean 0.0050 0.015, 0.024; mean 0.019	na	3
USA, (Watsonville, CA), 1997, Button	SC 480	0.063	19 25 33 42	0.01, 0.02; mean 0.02 0.04, 0.05; mean 0.04 <0.01 (2); mean <0.01 0.05 (2); mean 0.05	<0.01 (2); mean <0.01 <0.01 (2); mean <0.01 <0.01 (2); mean <0.01 <0.01 (2); mean <0.01	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005 0.010, 0.091; mean 0.050 0.012, 0.015; mean 0.014	na	3
USA, (Tempel, PA), 1997, Lambert 900	WP 250	0.063	16 23 33 41	0.06, 0.07; mean 0.06 0.06, 0.07; mean 0.06 0.04 (2); mean 0.04 <0.01 (2); mean <0.01	0.02 (2); mean 0.02 0.02, 0.03; mean 0.02 0.02 (2); mean 0.02 <0.01 (2); mean <0.01	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005 <0.005 (2); mean <0.005 <0.005, 0.0059; mean 0.0054	na	3
USA, (Tempel, PA), 1997, Lambert 900	SC 480	0.063	16 23 33 41	0.14 (2); mean 0.14 0.05 (2); mean 0.05 0.03 (2); mean 0.03 0.02 (2); mean 0.02	0.04 (2); mean 0.04 0.03 (2); mean 0.03 0.03 (2); mean 0.03 0.02 (2); mean 0.02	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005 <0.005 (2); mean <0.005 0.0065, 0.010; mean 0.0082	na	3
USA, (Tempel, PA), 1996, Amycel 2600	WP 250	0.068	19 27 36 43	0.09, 0.13; mean 0.11 0.02, 0.03; mean 0.02 0.02 (2); mean 0.02 <0.01, 0.03; mean 0.02	<0.01 (2); mean <0.01 <0.01 (2); mean <0.01 <0.01 (2); mean <0.01 <0.01 (2); mean <0.01	0.01, 0.02; mean 0.02 0.02 (2); mean 0.02 0.04, 0.05; mean 0.04 0.06, 0.08; mean 0.07	0.28, 0.29; mean 0.28 0.78, 0.81; mean 0.80 1.2, 1.5; mean 1.3 2.3, 2.6; mean 2.5	4
USA, (Tempel, PA), 1996, Amycel 2600	SC 480	0.068	19 27 36 43	0.06, 0.08; mean 0.07 0.03, 0.04; mean 0.04 0.01, 0.02; mean 0.02 0.03 (2); mean 0.03	<0.01 (2); mean <0.01 <0.01 (2); mean <0.01 <0.01 (2); mean <0.01 <0.01 (2); mean <0.01	<0.01 (2); mean <0.01 0.02, 0.03; mean 0.02 0.06, 0.07; mean 0.06 0.05, 0.07; mean 0.06	0.27, 0.30; mean 0.28 1.1, 1.2; mean 1.1 1.8, 2.1; mean 2.0 1.7, 3.0; mean 2.3	4
USA, (Gilroy, CA), 1996, A. bisporus	WP 250	0.25	16 23 33 45	2.6, 3.8; mean 3.2 2.7, 6.2; mean 4.5 1.4, 1.9; mean 1.6 0.32, 2.0; mean 1.1	<0.01, 0.02; mean 0.02 0.03, 0.05; mean 0.04 0.02 (2); mean 0.02 0.02 (2); mean 0.02	0.20, 0.30; mean 0.25 0.31, 0.42; mean 0.36 0.14, 0.29; mean 0.22 0.04, 0.12; mean 0.08	0.03 (2); mean 0.03 0.05 (2); mean 0.05 0.03, 0.04; mean 0.04 0.02, 0.04; mean 0.03	4
USA, (Gilroy, CA), 1996, A. bisporus	SC 480	0.25	16 23 33	5.1, 6.1; mean 5.6 1.0, 1.5; mean 1.2 0.77, 1.1;	0.03, 0.05; mean 0.04 0.02 (2); mean 0.02 0.02 (2);	0.42, 0.45; mean 0.44 0.14, 0.20; mean 0.17 0.08, 0.10;	0.05 (2); mean 0.05 0.03, 0.05; mean 0.04 0.05, 0.06;	4

Location, year, variety	Form	kg ai/hl	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	PCA, mg/kg	DFBA, mg/kg	Ref
				mean 0.92	mean 0.02	mean 0.09	mean 0.06	
			45	0.42, 0.45; mean 0.44	0.02, 0.04; mean 0.03	0.06, 0.18, mean 0.12	0.03, 0.04; mean 0.04	
Single casing treatment (10 kg ai/ha) plus second insecticide Apex 5E								
USA, (Tempel, PA), 1996, Amycel 2600	WP 250	0.068	37	0.19, 0.23; mean 0.21	<0.01 (2); mean <0.01	0.02 (2); mean 0.02	0.64, 0.70; mean 0.67	4
			45	0.03, 0.10; mean 0.06	<0.01 (2); mean <0.01	0.01, 0.02; mean 0.02	0.94, 1.1; mean 1.0	
			54	0.03, 0.05; mean 0.04	<0.01 (2); mean <0.01	0.04, 0.05; mean 0.04	2.3, 3.7; mean 3.0	
			61	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.03, 0.04; mean 0.04	2.9, 3.5; mean 3.2	
USA, (Gilroy, CA), 1996, A. bisporus	WP 250	0.25	29	2.8, 7.7; mean 5.3	0.02, 0.05; mean 0.04	0.32, 0.37; mean 0.34	0.04, 0.07; mean 0.06	4
			36	3.4, 4.4; mean 3.9	0.05 (2); mean 0.05	0.22, 0.38; mean 0.30	0.06, 0.08; mean 0.07	
			46	1.2, 1.6; mean 1.4	0.02 (2); mean 0.02	0.09, 0.11; mean 0.10	0.04 (2); mean 0.04	
			58	0.50, 2.3; mean 1.4	0.01, 0.04; mean 0.02	0.04, 0.11; mean 0.08	0.04 (2); mean 0.04	
Single casing treatment (15 kg ai/ha)								
Australia, Rydalmere, 1992, Off-white hybrid	WP 250	0.0075	17	0.029	0.011	na	0.55	5
			24	0.031	0.016	na	0.78	
			29	0.028	0.021	na	1.3	
Compost treatment (5 kg ai/ha) followed by casing treatment (5 kg ai/ha) 12 days later								
The Netherlands, Beesel, 1977; Soumycel 76	WP 250	ns	21	0.040	0.019	na	0.27	6
			28	0.014	0.011		<0.01	
			35	0.011	0.019		0.24	
			42	<0.01	0.015		0.16	
			49	0.015	0.018		0.14	
Compost treatment (10 kg ai/ha) followed by casing treatment (10 kg ai/ha) 11-13 days later								
The Netherlands, Horst, 1976, SOM 53	WP 250	ns	21	0.020	<0.02	na	0.54	7
			27	<0.02	<0.02		0.66	
			35	<0.02	0.035		0.64	
			43	0.036	0.030		0.54	
			49	<0.02	<0.02		0.80	
The Netherlands, Beesel, 1976, SOM 11	WP 250	ns	18	0.061	<0.02	na	0.23	7
			35	<0.02	<0.02		0.24	
			48	<0.02	<0.02		0.27	
The Netherlands, Horst, 1976, SOM 33	WP 250	ns	23	<0.03	0.023	na	0.93	8
			28	<0.03	0.048		1.0	
			35	<0.03	0.046		0.88	
			42	0.032	0.035		0.70	
The Netherlands, Melderslo, 1976; A. bitorquis K32	WP 250	ns	21	0.084	na	na	na	8
			45	0.28	0.065		0.13	
			55	0.12	0.044		0.082	
Compost treatment (49 kg ai/ha) followed by casing treatment (10 kg ai/ha) 13-16 days later								
USA, (Watsonville, CA), 1997, Button	WP 250	0.30[1] 0.061[2]	19	0.03 (2); mean 0.03	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005	na	3
			25	0.08 (2); mean 0.08	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005		
			33	0.02 (2); mean 0.02	0.01 (2); mean 0.01	<0.005 (2); mean <0.005		
			42	0.07 (2); mean 0.07	<0.01 (2); mean <0.01	0.0081, 0.014; mean 0.011		
USA, (Watsonville, CA), 1997, Button	SC 480	0.30[1] 0.061[2]	19	0.02, 0.03; mean 0.02	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005	na	3
			25	0.10 (2); mean 0.10	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005		
			33	0.01 (2); mean 0.01	0.02 (2); mean 0.02	<0.005, 0.0063; mean 0.0056		
			42	0.07 (2); mean 0.07	<0.01 (2); mean <0.01	0.0091, 0.010; mean 0.0096		

Location, year, variety	Form	kg ai/hl	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	PCA, mg/kg	DFBA, mg/kg	Ref
USA, (Tempel, PA), 1996, Amycel 2600	WP 250	0.80^1 0.066^2	19	0.03, 0.05; mean 0.04	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.36, 0.39; mean 0.38	4
			27	0.02, 0.04; mean 0.03	<0.01 (2); mean <0.01	0.01, 0.02; mean 0.02	0.78, 0.81; mean 0.80	
			36	0.01, 0.02; mean 0.02	<0.01 (2); mean <0.01	0.02, 0.04; mean 0.03	1.4 (2); mean 1.4	
			43	<0.01, 0.01; mean 0.01	<0.01 (2); mean <0.01	0.02, 0.03; mean 0.02	1.5, 1.7; mean 1.6	
USA, (Tempel, PA), 1996, Amycel 2600	SC 480	0.80^1 0.066^2	20	0.07, 0.13; mean 0.10	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.39, 0.41; mean 0.40	4
			28	0.02, 0.03; mean 0.02	<0.01 (2); mean <0.01	0.01, 0.02; mean 0.02	0.94, 1.4; mean 1.2	
			36	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.03, 0.04; mean 0.04	1.2, 1.5; mean 1.3	
			43	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	0.02, 0.03; mean 0.02	1.6, 1.7; mean 1.6	
USA, (Tempel, PA), 1997, Lambert 900	WP 250	1.20^1 0.061^2	16	0.03, 0.04; mean 0.04	0.01 (2); mean 0.01	<0.005 (2); mean <0.005	na	3
			23	0.04 (2); mean 0.04	0.03 (2); mean 0.03	<0.005 (2); mean <0.005		
			33	0.02 (2); mean 0.02	0.03 (2); mean 0.03	0.0059, 0.0069; mean 0.0060		
			41	0.04 (2); mean 0.04	0.03 (2); mean 0.03	0.0068, 0.0080; mean 0.0074		
USA, (Tempel, PA), 1997, Lambert 900	SC 480	1.20^1 0.061^2	16	0.05 (2); mean 0.05	0.03 (2); mean 0.03	<0.005 (2); mean <0.005	na	3
			23	0.03, 0.04; mean 0.04	0.03 (2); mean 0.03	<0.005 (2); mean <0.005		
			33	0.02 (2); mean 0.02	0.02, 0.03; mean 0.02	0.011 (2); mean 0.011		
			41	0.02 (2); mean 0.02	0.02, 0.04; mean 0.03	0.015, 0.017; mean 0.016		
USA, (Gilroy, CA), 1996, A. bisporus	WP 250	1.20^1 0.25^2	16	4.0, 8.2 mean 6.1	0.02 (2); mean 0.02	0.32, 0.51; mean 0.42	0.03, 0.05; mean 0.04	4
			23	4.2, 9.4; mean 6.8	0.05 (2); mean 0.05	0.32, 0.94; mean 0.63	0.04 (2); mean 0.04	
			33	1.6, 2.8; mean 2.2	0.03, 0.04; mean 0.04	0.14, 0.19; mean 0.16	0.04, 0.06; mean 0.05	
			45	0.07, 1.8; mean 0.96	0.02, 0.04; mean 0.03	0.03, 0.08; mean 0.06	0.04, 0.05; mean 0.04	
USA, (Gilroy, CA), 1996, A. bisporus	SC 480	1.20^1 0.25^2	17	2.1, 7.3; mean 4.7	0.02, 0.06; mean 0.04	0.22, 0.47; mean 0.34	0.06, 0.08; mean 0.07	4
			24	2.2, 2.4; mean 2.3	0.03 (2); mean 0.03	0.19, 0.27; mean 0.23	0.07, 0.08; mean 0.08	
			34	0.84, 0.89; mean 0.86	0.02 (2); mean 0.02	0.09, 0.14; mean 0.12	0.05, 0.09; mean 0.07	
			46	0.48, 0.49; mean 0.48	0.02, 0.03; mean 0.02	0.06, 0.08; mean 0.07	0.03, 0.05; mean 0.04	
Compost treatment (20 kg ai/ha) followed by casing treatment (20 kg ai/ha) 12 days later								
The Netherlands, Horst, 1976, SOM 53	WP 250	ns	21	0.039	<0.02	na	0.51	7
			27	0.061	<0.02		0.90	
			35	0.061	0.045		0.70	
			43	0.033	0.044		0.32	
			49	0.050	0.063		0.92	
The Netherlands, Horst, 1976, SOM 33	WP 250	ns	23	<0.03	0.029	na	1.5	8
			28	0.032	0.063		1.7	
			35	0.040	0.069		1.1	
			42	<0.03	0.067		0.82	
Compost treatment (30 kg ai/ha) followed by casing treatment (30 kg ai/ha) 13 days later								
The Netherlands, Horst, 1975; A bisporus Sinden A$_1$; Sinden A$_2$	WP 250	ns	21	0.076	na	na	na	9
			28	0.25				
			40	0.12				
			43	0.092				
			48	0.13				

Location, year, variety	Form	kg ai/hl	PHI, days	diflubenzuron, mg/kg	CPU, mg/kg	PCA, mg/kg	DFBA, mg/kg	Ref
Compost treatment (40 kg ai/ha) followed by casing treatment (40 kg ai/ha) 12 days later								
The Netherlands, Horst, 1976, SOM 53	WP 250	ns	21 27 35 43 49	0.082 0.12 0.089 0.064 0.059	<0.02 <0.02 <0.02 0.044 0.044	na	1.1 1.1 0.87 0.88 1.1	[7]
The Netherlands, Horst, 1976, SOM 33	WP 250	ns	23 28 35 42	0.032 0.052 0.072 0.040	0.036 0.076 0.10 0.076	na	1.9 1.4 2.3 1.3	[8]
Compost treatment (90 kg ai/ha) followed by casing treatment (90 kg ai/ha) 13 days later								
The Netherlands, Horst, 1975; A bisporus Sinden A_1; Sinden A_2	WP 250	ns	21 28 40 43 48	0.13 0.088 0.32 0.36 0.43	na	na	na	[9]

ns: not specified
na: not analysed
[1] spray concentration for compost treatment
[2] spray concentration for casing treatment
[3] RP-97004, Gaydosh 1998a, GLP. Spray volume 16296 l/ha (CA) and 4074 l/ha for compost and 16296 l/ha at casing (PA). Spray can pressurised with carbon dioxide and fitted with a hand-held boom (CA) or a hand held sprayer (PA) . Sampling in Z pattern from each tray (CA) or at random (PA); 0.45-2.2 kg (>24 mushrooms)/sample. Controlled indoor environment (14-22°C; plot size 3.9-12.3 m^2). Storage at -20°C for 34-76 days for diflubenzuron, 29-71 days for CPU and 53-146 days for PCA.
[4] RP-96015, Gaydosh 1999c, GLP, some applications were in combination with the insecticide Apex 25E (0.45 oz ai/1000 sq. ft). Spray volume 4074 l/ha (CA), or 6111 l/ha for compost (PA) and 15074 l/ha for casing (PA). Back pack sprayers pressurised with carbon dioxide and fitted with a hand-held boom (CA) or a hose-end watering wand for broadcast application (PA). Controlled indoor environment (13-32°C; plot size 2.0-4.2 m^2). Sampling from all areas of each plot; 0.45-1.8 kg/sample (24-60 mushrooms/sample). Storage at -20°C for 25-99 days for diflubenzuron, 33-112 days for CPU and 37-105 days for PCA and 30-120 days for DFBA.
[5] DI-9441, Allan and Thus, 1996, non-GLP. The suspension was mixed through the casing with a casing mixer, spray volume 200000 l/ha. Controlled indoor environment (15°C, plot size 0.2 m^2), storage at -10°C for 15-45 days for diflubenzuron, CPU and DFBA. Mushrooms (1 kg/sample) were sampled by standard mushroom harvesting procedures.
[6] DI-3409, De Wilde, 1979a, non-GLP. Sampling at random, 2.5 kg/sample. Samples were stored at –20°C (time not stated). Spray equipment, climatic conditions and plot size not stated.
[7] DI-1309, De Wilde, 1978b, non-GLP. 1 kg samples taken at random. Compost and casing 12 pooled samples taken at random with an auger. Equipment, climatic conditions, plot size, and storage conditions not stated.
[8] DI-3410, De Wilde, 1978a, non-GLP. Spray equipment, climatic conditions, plot size, sampling and storage conditions not stated.
[9] DI-2726, Buisman *et al.*, 1976a, non-GLP, mushrooms were surface sprayed. Equipment, climatic conditions, plot size, sampling and storage conditions not stated. Mushrooms were washed before analysis. Thermostat temperature between 2nd – 3rd flush was too high (30°C) resulting in dried up mushrooms at 40-day PHI.

Pulses and legume animal feeds (groups 015 and 050)

Results of trials on peas and soya beans are shown in Table 126.

In trials in the UK in 1978 (De Wilde, 1980, non-GLP) on peas samples were analysed for diflubenzuron by method LAI 3-86-4 (no description available). Residues were not corrected for concurrent method recoveries (73%-100%) or matrix interferences (<0.003 mg/kg diflubenzuron).

In trials in the USA in 1996 (Gaydosh, 2000f, GLP) on soya beans samples were analysed for diflubenzuron by HPLC method 3-86-13. Residues were corrected for concurrent recoveries if <100% (67%-250%), but not for matrix interferences (<0.05 mg/kg in seed, max 0.31 mg/kg in forage, max 1.0 mg/kg in hay, Table 53). Because of matrix interferences results below 1 mg/kg in forage and 3 mg/kg in hay are not considered valid.

Table 126. Residues of diflubenzuron in peas and soya bean forage, hay and seed.

Location, year, variety	Form.	kg ai/ha	kg ai/hl	interval, days	PHI, days	sample	diflubenzuron, mg/kg	Ref
peas								
UK, Terrington St. John, 1978, Marc	WP 250	2x 0.25	ns	ns	50	ns	0.023	[3]
UK, Terrington St. John, 1978, Marc	WP 250	2x 0.30	ns	ns	50	ns	0.022	[3]
soya bean								
USA, (Senetobia, MS), 1996, Pioneer 9592	WP 250	1x0.074 1x0.077	2x0.078	113	32[1] 73[1] 22[2]	forage hay seed	<0.05, 0.14; mean 0.10 0.40, 0.45; mean 0.42 <0.05 (2); mean <0.05	[4]
USA, (Senetobia, MS), 1996, Pioneer 9592	SC 240	2x0.075	2x0.078	113	32[1] 73[1] 22[2]	forage hay seed	0.05, 0.06; mean 0.06 0.55, 0.84; mean 0.70 <0.05 (2); mean <0.05	[4]
USA, (Lamberton, MN), 1996, Parker	WP 250	2x0.076	2x0.041	48	28[1] 44[1] 21[2]	forage hay seed	0.60, 0.80; mean 0.70 1.7, 1.8; mean 1.8 <0.05 (2); mean <0.05	[4]
USA, (Lamberton, MN), 1996, Parker	SC 240	2x0.073	2x0.039	48	28[1] 44[1] 21[2]	forage hay seed	0.73, 0.68; mean 0.70 1.6 (2); mean 1.6 <0.05 (2); mean <0.05	[4]

[1] Forage was harvested 28-32 days after first application, at bloom to early pod formation and hay 44-73 days after first application and dried for 3-8 days in the field before sampling

[2] Soya bean seeds harvested at maturity 21-22 days after second application.

[3] DI-201, De Wilde, 1980, non-GLP, harvest August, storage at –20°C (time not stated). Equipment, soil, weather conditions, plot size and sampling not stated.

[4] RP-96014, Gaydosh, 2000f, GLP, first application at the node 3-4 stage. Back pack CO2 sprayer (Senetobia) with spray volume 93 l/ha for each application; tractor mounted compressed air sprayer (Lamberton) volume 187 l/ha for each application. No unusual weather conditions (Jun-Oct, temp –4-36°C, 13-60 cm rain). USDA soil type loam (Lamberton), silt loam (Senetobia). Harvest Jul-Oct. Plot size 186-282 m^2; 2 field samples/plot; 1.1 kg forage/sample; 1.1 kg seed/sample; 0.68 kg hay/sample. Soya bean forage and hay samples were cut within 4-5 inches of the ground; for the harvest of soya bean seeds, plants were broken at ground level by hand or were cut with a tractor-operated sickle mower. Soya bean seeds were separated from the plants by a small-plot thresher. Storage at <-20°C for 53-161 days.

Cereal grains (group 020)

Results of trials on rice (all USA) are shown in Table 127.

Samples were separated into grain (kernels plus hull) and straw with a thresher. Straw samples were obtained from the stubble left after the harvest or from what was left lying after separating the grain.

The samples were analysed by GC-ECD method LAI 3-86-6 for diflubenzuron, GC-ECD method LAI 3-86-9 for CPU, and CARDC-1248-PCA in the RP95016 trials and PTRL 645W in all other trials for PCA. Residues were not corrected for concurrent method recoveries (60%-142% for diflubenzuron, 71-119% for CPU, 83%-125% for PCA) or matrix interferences (all ≤LOQ), (Tables 74, 83, 87 and 93). The storage stabilities of diflubenzuron and CPU were not validated beyond 1 month, when they were stable, because of analytical problems. In 6 of the 21 trials (references 3, 4 and 5) the storage time before diflubenzuron determination was 162-388 days so these trials were not evaluated. In the remaining 15 trials the storage time was 9-76 days before diflubenzuron determination and these were evaluated.

Residues in rice grain from Cleveland (1996 trials, high rate) were abnormally high and the authors could not give any reasonable explanation.

Table 127. Residues of diflubenzuron and metabolites in rice grain and straw, USA.

Location, year, variety	Form.	No	kg ai/ha	kg ai/hl	PHI, days	sample	diflubenzuron, mg/kg	CPU, mg/kg	PCA, mg/kg	Ref
LV sprays										
Rayne, LA, 1998, Cypress	SC 240	2	0.14	0.10	93	grain	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	1
						straw	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	
Rayne, LA, 1998, Cypress	SC 240	2	0.14	0.10	93	grain	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	1
						straw	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	
Tunica, MS, 1996, Lemont	SC 240	1	0.28	0.30	108	grain	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	2
						straw	0.01, 0.02; mean 0.02	<0.01 (2); mean <0.01	0.006, 0.007; mean 0.006	
Hamilton City, CA, 1996, M202	WP 250	1	0.28	0.30	105	grain	<0.01 (2); mean <0.01	0.001 (2); mean 0.001	<0.005 (2); mean <0.005	2
						straw	<0.01 (2); mean <0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005	
Heth, AR, 1996, Bengal	SC 240	1	0.28	0.30	97	grain	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	2
						straw	0.01 (2); mean 0.01	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005	
Senetobia, MS, 1995, Jackson	WP 250	1	0.28	0.30	91	grain	<0.01 (2); mean <0.01	na	<0.025 (2); mean <0.025	3
						straw	0.06, 0.07; mean 0.06			
W. Memphis, AR, 1996, Bengal	SC 240	1	0.28	0.30	91	grain	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	2
						straw	0.13, 0.15; mean 0.14	<0.01, 0.02; mean 0.02	0.005, 0.007; mean 0.006	
Cleveland, MS, 1996, Cypress	SC 240	1	0.28	0.30	85	grain	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	2
						straw	0.39, 0.57; mean 0.48	0.02 (2); mean 0.02	0.017, 0.021; mean 0.019	
Brookshire, TX, 1996, Lemont	WP 250	1	0.28	0.30	83	grain	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	2
						straw	0.05, 0.08; mean 0.06	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005	
Brookshire, TX, 1996, Lemont	SC 240	1	0.28	0.30	83	grain	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	2
						straw	0.24, 0.26; mean 0.25	<0.01 (2); mean <0.01	<0.005 (2), mean <0.005	
Washington, LA, 1996, Cypress	SC 240	1	0.28	0.30	77	grain	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	2
						straw	0.34, 0.46; mean 0.40	<0.01 (2); mean <0.01	0.012, 0.017; mean 0.014	
Pattison, TX, 1996, Lemont	SC 240	1	2.2	0.9	82	grain	<0.01 (2); mean <0.01	0.006, 0.008; mean 0.007	<0.005 (2); mean <0.005	4
						straw	1.8 (2); mean 1.8	0.05, 0.07; mean 0.06	0.025 (2); mean 0.025	
Cleveland, MS, 1996, Cypress	SC 240	1	2.2	1.2	85	grain	0.26, 0.87; mean 0.56	0.006; 0.012; mean 0.009	<0.005, 0.005; mean 0.005	4
						straw	3.5, 3.6; mean 3.5	0.03, 0.04; mean 0.04	0.049, 0.053; mean 0.051	

Location, year, variety	Form.	No	kg ai/ha	kg ai/hl	PHI, days	sample	diflubenzuron, mg/kg	CPU, mg/kg	PCA, mg/kg	Ref
Tunica, MS, 1995, Lemont	WP 250	1	2.2	2.4	94	grain straw	<0.01, 0.02; mean 0.02 0.02, 0.07; mean 0.04	<0.001 (2); mean <0.001 <0.01 (2); mean <0.01	<0.005 (2); mean <0.005 0.008, 0011; mean 0.010	5
LV aerial spray										
Hamilton City, CA, 1996, M202	SC 240	1	0.28	0.30	105	grain straw	<0.01 (2); mean <0.01 <0.01 (2); mean <0.01	0.002 (2); mean 0.002 <0.01 (2); mean <0.01	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	2
Pleasant Grove, CA, 1998, Akita Komachi	SC 240	1	0.28	0.30	101	grain straw	<0.01 (2); mean <0.01 <0.01 (2); mean <0.01	<0.001 (2); mean <0.001 <0.001 (2); mean <0.001	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	1
Live Oak, CA, 1995, M202	WP 250	1	0.28	0.30	100	grain straw	<0.01 (2); mean <0.01 0.01, 0.02; mean 0.02	na	<0.025 (2); mean <0.025 na	3
Brookshire, TX, 1996, Lemont	SC 240	1	0.28	0.55	83	grain straw	<0.01 (2); mean <0.01 0.13, 0.17; mean 0.15	<0.001 (2); mean <0.001 <0.01 (2); mean <0.01	<0.005 (2); mean <0.005 0.007, 0.008; mean 0.008	2
Beaumont, TX, 1998, Cypress	SC 240	1	0.28	ns	83	grain straw	<0.01 (2); mean <0.01 <0.01 (2); mean <0.01	<0.001 (2); mean <0.001 <0.001 (2); mean <0.001	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	1
Rayne, LA, 1998, Drew	SC 240	ns	0.28 total	ns	ns	grain straw	<0.01 (2); mean <0.01 <0.01 (2); mean <0.01	<0.001 (2); mean <0.001 <0.001 (2); mean <0.001	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	1
Winters, CA, 1995, M-202	WP 250	1	2.2	2.4	115	grain straw	<0.01 (2); mean <0.01 0.03, 0.04; mean 0.04	<0.001 (2); mean <0.001 <0.01 (2); mean <0.01	<0.005 (2); mean <0.005 0.006 (2); mean 0.006	5

[1] RP-98029, Gaydosh and Puhl, 1998, GLP, single low volume aerial spray or a double low volume ground spray (with a 6-7 day interval with a back pack sprayer) with spray volumes of 93-140 l/ha at 3- to 4-leaf stage on post permanent flood seeded rice (Oryza sativa). No unusual weather conditions (May-July), except in CA where the spring was cooler than normal, causing delayed planting and slow early plant growth and in last 6 weeks the temperature was higher than normal, causing stress. Soil not stated, plot size 8.4 m^2 – 6.5 ha; 2 field samples/plot; sample size not stated. Harvest Aug/Sept. Rice storage frozen for 9-51 days for diflubenzuron, 9-58 days for CPU, 16-56 days for PCA (temperature not stated).

[2] RP-96012, Gaydosh *et al.*, 1997a, GLP, low volume aerial spray (aircraft boom sprayer 51-93 l/ha) or low volume ground spray (93 l/ha). Applications 2 weeks post emergence (Hamilton City) or 10 days after permanent flood (other sites). No unusual weather conditions (May-Sept; temp 9-43°C; rainfall 0.8-46 cm), except at Cleveland (MS) and Heth (AR) where the growing season was drier than normal. USDA soil type loam (Hamilton City, pH 7.4, 0.8% om, CEC 12.3), sandy loam (Brookshire, pH 4.9, 1.1% om, CEC 6.9), clay (Tunica, pH 5.6, 2.1% om, CEC 23.2 and Heth, pH 6.6, 1.6% om, CEC 28.2), silt loam (Cleveland, pH 6.6, 2.4% om, CEC 16.8), silty clay loam (West Memphis, pH 6.5, 1.1% om, CEC 18.1 and Washington, pH 5.3, 2.3% om, CEC=17.8). Harvest Sept/Oct. Rice was sampled randomly from 12 areas of the plot by severing 6 inches above the soil, plot size >0.1 ha; 2 field samples/plot; 2.3 kg grain/sample; 0.9 kg straw/sample; 178 ml water/sample. Storage of rice at <-20°C for 14-76 days for diflubenzuron, 153-233 days for CPU, 174-228 days for PCA.

[3] RP-95016, Gaydosh, 1996, GLP, low volume aerial spray 2 weeks post-emergence (Live Oak, aircraft boom sprayer) or low volume spray 10 days after permanent flood (Senetobia, back pack sprayer), spray volume 93 l/ha. No unusual weather conditions (Jun-Sept; temp 8-41°C; rainfall 8-36 cm), except in Senetobia where growing season was cooler and wetter than normal in early to mid season, and hotter and drier than normal later in the season. USDA soil type clay (Live Oak, pH 7.1, 1.8% om, CEC 43.3; Senetobia, pH 5.4, 2.0% om, CEC 22.7). Harvest Sept. Rice sampled randomly by severing 6 inches above soil or by mowing, plot size >0.1 ha; 2 field samples/plot; 11 kg grain/sample; 5.4 kg straw/sample; 178 ml water/sample. Storage at –5°C to –20°C for 162-210 days for diflubenzuron and PCA in rice.

[4] RP-96013, Gaydosh *et al.*, 1997b, GLP, low volume spray (187-262 l/ha; CO_2 back pack sprayer) at 10 days after permanent flood (Cleveland) or 10 days after seeding (Pattison). In Cleveland the rainfall was slightly below normal and in Pattison the weather was drier than normal in May-Jul and slightly wetter than normal in Aug. (Jun-Sept; temp 16-37°C; rainfall 12-20 cm). Soil sandy loam (Pattison, pH 5.0, 1.1% om, CEC 6.9), silt loam (Cleveland, pH 6.8, 2.8% om, CEC 16.0). Harvest Sept. Rice was sampled at random, plot size 2787-4645 m^2; 2 field samples/plot; 23 kg grain/sample; 0.9 kg straw/sample). Storage of rice at <-20°C for 178-214 days for diflubenzuron, 217-237 days for CPU, 222-245 days for PCA.

[5] RP-95028, Gaydosh, 1998b, GLP, low volume spray 10 days after permanent flood (Tunica, CO_2 back pack sprayer) or low volume aerial spray 14 days post-emergence (Winters), both at an exaggerated rate of 2.2 kg ai/ha. Spray volume 93 l/ha. No unusual weather conditions (Jun-Oct; temp 6-41°C; rainfall 1-41 cm), except in Tunica where rainfall was above normal, temperatures slightly cooler than normal in May-June and weather hotter and drier than normal in August. USDA soil type clay (Winters, pH 5.4, 2.4% om, CEC 33.4; Tunica, pH 5.6, 3.3% om, CEC 25.3). Harvest Sept/Oct. Sampling at random, plot size 1700 m^2; 2 field samples/plot; 68-77 kg grain/sample; 5.4 kg straw/sample. Storage at <-20°C for 351-388 days for diflubenzuron, 499-542 days for CPU, 563-613 days for PCA.

Tree nuts (group 022)

Results of trials on walnuts, almonds and pecans are shown in Table 128.

Walnuts were shelled and only the kernels analysed. Almonds were air-dried in the field and separated into kernels, shells and hulls, by separators. Pecans were mechanically separated into kernels, shells and hulls at a local cracker/sheller facility. The samples were analysed for diflubenzuron by GC-ECD method RES013 for walnuts and HPLC method LAI 3-86-13 for almonds and pecans, HPLC-MS method H for CPU and method PTRL 645W for PCA. Residues were corrected for recoveries in walnuts and pecans if <100% (70%-94% for diflubenzuron, 69%-90% for CPU, 75%-120% for PCA), but not for recoveries in almonds (80%-114% for diflubenzuron, 80%-130% for CPU, 94%-117% for PCA) nor for matrix interferences (all <LOQ) (Tables 53 and 77 for diflubenzuron, Table 73 for CPU and Table 93 for PCA). CPU was <0.005 mg/kg in kernels and <0.01 mg/kg in hulls in all samples and so results were not tabulated.

In trials on walnuts in France in 1999 samples were analysed for diflubenzuron by GC-ECD method RES013. Residues were not corrected for recoveries (70%-86%) or matrix interferences.

Table 128. Residues of diflubenzuron and PCA in tree nuts, USA.

Location, year, variety	Form.	kg ai/ha	kg ai/hl	Interval, days	PHI, days	Sample	diflubenzuron, mg/kg	PCA, mg/kg	Ref
Walnuts									
Hughson, (CA), 1988, Ashley	WP 250	4x0.56	4x0.015	27, 27, 36	28	kernels	<0.05 (2); mean <0.05	na	[1]
Waterford, (CA), 1988, Ashley	WP 250	4x0.56	4x0.015	25, 35, 29	28	kernels	<0.05 (2); mean <0.05	na	[1]
Kerman, (CA), 1988, Hartley	WP 250	4x0.56	4x0.015	35, 27, 36	28	kernels	<0.05 (2); mean <0.05	na	[1]
S. France, Pessoulens, 32380 Gers, 1999, Franquette	SC 150	1x0.10	1x0.0099	-	28	kernels	<0.05	na	[2]
S. France, Douelle, 46140 Lot, 1999, Franquette	SC 150	1x0.10	1x0.0099	-	28	kernels	<0.05	na	[2]
Almonds	-	-	-		-	-	-	-	-
Madera, (CA), 1998, Nonpareil	WP 250	1x0.56 2x0.28 1x0.56	3x0.060 1x0.12	21, 81, 86	28	kernels hulls	<0.05 (2); mean <0.05 2.1 (2); mean 2.1	<0.005 (2); mean <0.005 <0.005, 0.0082; mean 0.0066	[3]
Kerman, (CA), 1998, Nonpareil	WP 250	1x0.57 1x0.27 1x0.29 1x0.56	1x0.060 1x0.059 1x0.061 1x0.12	23, 73, 70	28	kernels hulls	<0.05 (2); mean <0.05 2.3 (2); mean 2.3	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	[3]
Reedley, (CA), 1998, Butte	WP 250	1x0.56 2x0.28 1x0.56	1x0.060 2x0.059 1x0.12	21, 79, 83	28	kernels hulls	<0.05 (2); mean <0.05 2.9, 3.6; mean 3.3	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	[3]
Manteca, (CA), 1998, Nonpareil	WP 250	1x0.56 2x0.28 1x0.56	2x0.060 1x0.061 1x0.12	21, 78, 89	28	kernels hulls	<0.05 (2); mean <0.05 0.97, 1.1; mean 1.0	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	[3]

Location, year, variety	Form.	kg ai/ha	kg ai/hl	Interval, days	PHI, days	Sample	diflubenzuron, mg/kg	PCA, mg/kg	Ref
Ripon, (CA), 1998, Nonpareil	WP 250	1x0.81 1x0.49 1x0.28 1x0.55	2x0.060 1x0.059 1x0.12	21, 83, 84	28	kernels hulls	<0.05 (2); mean <0.05 1.5, 1.6; mean 1.5	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	3
Reedley, (CA), 1998, Butte	WG 800	1x0.57 2x0.28 1x0.56	1x0.060 1x0.059 1x0.060 1x0.12	21, 79, 83	28	kernels hulls	<0.05 (2); mean <0.05 3.4, 5.5; mean 4.5	<0.005 (2); mean <0.005 0.0052, 0.0074; mean 0.0063	3
Reedley, (CA), 1998, Butte	SC 240	1x0.56 2x0.28 1x0.56	1x0.059 1x0.060 1x0.059 1x0.12	21, 79, 83	28	kernels hulls	<0.05 (2); mean <0.05 2.9, 3.1; mean 3.0	<0.005 (2); mean <0.005 <0.005, 0.0067; mean 0.0059	3
Pecans									
Finleyson, (GA), 1999, Stuarts	SC 240	1x0.56 2x0.28 1x0.56	1x0.060 1x0.058 1x0.062 1x0.12	21, 20, 154	28	kernels hulls	<0.05 (2); mean <0.05 0.52, 0.64; mean 0.58	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	4
Finleyson, (GA), 1999, Stuarts	WP 250	1x0.56 2x0.28 1x0.56	1x0.061 1x0.057 1x0.062 1x0.12	21, 20, 154	28	kernels hulls	<0.05 (2); mean <0.05 0.21, 0.23; mean 0.22	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	4
Finleyson, (GA), 1999, Stuarts	WG 800	1x0.56 2x0.28 1x0.56	1x0.061 1x0.057 1x0.062 1x0.12	21, 20, 154	28	kernels hulls	<0.05 (2); mean <0.05 0.31, 0.37; mean 0.34	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	4
Eastman, (GA), 1999, Desirable	SC 240	1x0.57 2x0.28 1x0.55	1x0.061 1x0.059 1x0.061 1x0.12	21, 14, 150	28	kernels hulls	<0.05 (2); mean <0.05 0.89, 1.4; mean 1.1	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	4
Opelousas, (LA), 1999, Melrose	SC 240	1x0.56 2x0.28 1x0.56	3x0.059 1x0.12	11, 34, 157	28	kernels hulls	<0.05 (2); mean <0.05 0.33, 0.40; mean 0.37	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	4
Opelousas, (LA), 1999, Melrose	WP 250	1x0.56 2x0.28 1x0.56	3x0.059 1x0.12	11, 34, 157	28	kernels hulls	<0.05 (2); mean <0.05 0.37, 0.76; mean 0.56	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	4
Opelousas, (LA), 1999, Melrose	WG 800	1x0.56 2x0.28 1x0.56	3x0.059 1x0.12	11, 34, 157	28	kernels hulls	<0.05 (2); mean <0.05 0.20, 0.33; mean 0.27	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	4
Duncan, (OK), 1999, Natives	SC 240	1x0.56 1x0.28 1x0.29 1x0.56	1x0.059 1x0.063 1x0.061 1x0.12	28, 22, 152	28	kernels	<0.05 (2); mean <0.05 0.098, 0.18; mean 0.14	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	4
Duncan, (OK), 1999, Natives	WP 250	1x0.56 2x0.28 1x0.56	1x0.059 1x0.063 1x0.061 1x0.12	28, 22, 152	28	kernels hulls	<0.05 (2); mean <0.05 0.065, 0.090; mean 0.078	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	4
Duncan, (OK), 1999, Natives	WG 800	1x0.56 1x0.28 1x0.29 1x0.56	1x0.059 1x0.063 1x0.062 1x0.12	28, 22, 152	28	kernels hulls	<0.05 (2); mean <0.05 <0.05 (2); mean <0.05	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	4
Rincon, (NM), 1999, Western Schley	SC 240	1x0.55 2x0.28 1x0.54	1x0.059 1x0.061 1x0.060 1x0.12	21, 28, 175	28	kernels hulls	<0.05 (2); mean <0.05 0.22, 0.33; mean 0.28	<0.005 (2); mean <0.005 <0.005 (2); mean <0.005	4

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[1] RP-88022, Gaydosh 1993a, GLP, first application after first bloom. Spray volume 3738 l/ha for each spray using airblast sprayers. No unusual weather conditions (May-Sept, temp -12-44°C; total rain not stated). Soil not stated, 12 trees per plot; plot size not stated; 2 field samples/plot; 1.4 kg kernels/sample. Harvest Sept. Storage at –15°C for 185-186 days.

[2] DI-11544, Anthony and Brown, 2000, GLP. Knapsack mist blower, spray volume 1009-1010 l/ha, application at ripening stage (BBCH 81), other pesticides used (sulfonate, copper oxide, copper sulfate, maneb and lambda-cyhalothrin, copper

hydroxide, mancozeb, diuron, terbuthylazine, glyphosate); more rainfall than normal (Sept, temp 5.0-32.0°C, total rain 79.6-99.8 mm); soil: clay (Pessoulens) or sandy silt loam (Douelle); tree height 4-6 m, 6 trees per plot; plot size 384-390 m^2; harvest Sept/Oct, mature samples (BBCH code 87-89) from 4 central trees, from all parts of the tree (top, bottom, exposed and covered by foliage), 1 kg/sample. Stored at –18°C 3-4 hours after harvest until extraction (25-26 days), extracts stored for 8 days at 4°C until analysis.

[3] RP-98003, Gaydosh 2000c, GLP, 4 applications: the first during dormancy, just before bud swell, the second at bloom to petal fall, the third during mid-May when almonds were small and the fourth 28 days before harvest. Spray volume 935 l/ha for the first application (including 19-56 l/ha dormant oil) and 467 l/ha for the remaining three applications. Tractor-mounted airblast sprayers. No unusual weather conditions (April-Sept, temp 0-42°C; total rain 15-35 cm), except at Reedley (CA) where the weather was wetter and cooler and almond maturity was delayed. USDA soil type sandy loam (Madera, Kerman), loamy coarse sand (Manteca, Ripon). Harvest Aug/Sept. Nut selection from all quadrants from inner trees and/or inner canopies of outer trees, 16-30 trees per plot; plot size 505-1350 m^2, 2 field samples/plot; 1.4 kg kernels/sample; 1.4 kg hulls/sample. Storage at < 0°C for 151 days for diflubenzuron, 269-312 days for CPU and 90-148 days for PCA.

[4] RP-99002, Gaydosh 2000d, GLP, 4 applications: the first during dormancy, just before bud swell (0.56 kg ai/ha; 213-252 days pre-harvest), the second at bloom to petal fall (0.28 kg ai/ha, 192-231 days pre-harvest), the third at early fruit set (0.28 kg ai/ha; 178-203 days pre-harvest) and the fourth 28 days before harvest (0.56 kg ai/ha). Spray volume 935 l/ha for the first application (including 18.7 l/ha dormant oil) and 467 l/ha for the remaining three applications. Tractor-mounted airblast sprayers. No unusual weather conditions (Mar-Oct, temp -7-40°C; total rain 20-64 cm). USDA soil type: sandy loam (Eastman), silt loam (Opelousas), silty clay loam (Duncan) or loamy sand (Rincon). Harvest Nov/Dec. Fruit selection from all quadrants from inner trees and/or inner canopies of outer trees, 8-9 trees per plot; plot size 752-3010 m^2; 2 field samples/plot; >5.6 kg whole pecans/sample. Storage at < 0°C for 43-92 days for diflubenzuron, 149-195 days for CPU, 119-178 days for PCA.

Oilseed (group 023)

Results of trials on cotton in the USA are shown in Table 129.

Cotton was ginned at each of the test sites in the 1993 trials, and harvested mechanically and separated batchwise into seed and gintrash, simulating commercial practice, in the 1995 trials (Gaydosh 1994a, 2000e). The samples were analysed for diflubenzuron by HPLV-UV method LAI 3-86-13 (1993) or GC-ECD method LAI 3-86-6 (1995). Residues were not corrected for concurrent method recoveries (70%-105%) or matrix interferences (<0.05 mg/kg). Because of the high LOQ (0.2 mg/kg diflubenzuron) and low recoveries from gintrash (60%-77%) in 1995, the gintrash results were disregarded.

Table 129. Residues of diflubenzuron in cotton after ground and aerial spray applications, USA.

Location, year, variety	Form	kg ai/ha; total	kg ai/hl	Interval, days	PHI, days	Sample	diflubenzuron, mg/kg	Ref
Low volume ground spray								
Hawkinsville, (GA), 1993, DES 119	WP 250	6x0.070; Σ 0.42	6x0.054	11, 9, 48, 10, 7	13	seed	0.10, 0.17; mean 0.14	1
Graingerbury, (AL), 1993, DPL 90	WP 250	6x0.070; Σ 0.42	6x0.037	10, 10, 42, 14, 7	14	seed	0.06, 0.07; mean 0.06	1
Hawkinsville, (GA), 1993, DES 119	SC 240	6x0.070; Σ 0.42	6x0.054	11, 9, 48, 10, 7	13	seed	0.13, 0.17; mean 0.15	1
Graingerbury, (AL), 1993, DPL 90	SC 240	6x0.070; Σ 0.42	6x0.037	10, 10, 42, 14, 7	14	seed	<0.05 (2); mean <0.05	1
Senetobia, (MS), 1995, DPL 50	SC 240	6x0.070; Σ 0.42	6x0.050	10, 10, 59, 23, 7	14	seed gintrash	<0.2 (2); mean <0.2 6.1, 7.9; mean 7.0	2
East Bernard, (TX), 1995, DPL 50	SC 240	6x0.070; Σ 0.42	6x0.063	10, 9, 36, 9, 10	14	seed gintrash	<0.2 (2); mean <0.2 7.5, 8.4; mean 8.0	2
Senetobia, (MS), 1995, DPL 50	WG 800	6x0.070; Σ 0.42	6x0.050	10, 10, 59, 23, 7	14	seed gintrash	<0.2 (2); mean <0.2 5.5, 9.7; mean 7.6	2
East Bernard, (TX), 1995, DPL 50	WG 800	6x0.070; Σ 0.42	6x0.063	10, 9, 36, 9, 10	14	seed gintrash	<0.2 (2); mean <0.2 7.4, 7.5; mean 7.4	2
ULV aerial spray								
Hawkinsville, (GA), 1993, DES 119	SC 240	6x0.070; Σ 0.42	6x6.0	11, 8, 51, 13, 7	14	seed	0.07; 0.08; mean 0.08	1
Graingerbury, (AL), 1993, DPL 90	SC 240	6x0.070; Σ 0.42	6x6.0	10, 10, 42, 14, 7	14	seed	0.06; 0.07; mean 0.06	1

[1] RP-93007, Gaydosh 1994a, GLP, three applications before and three after boll opening, from pin head square up to maturity. Low volume spray (121-187 l/ha) or ultra low volume aerial spray (1.2 l/ha); dissolved in crop or cotton seed oil. No unusual weather conditions (Jul-Oct, air temp 13-30°C; rain not stated). USDA soil type sand (Hawkinsville; pH 6.9, 0.5% om, CEC 4.1) or loamy sand (Graingerbury; pH 4.9, 1.3% om, CEC 1.8). Plot size not stated, 2 field samples per plot, 1.4 kg ginned cotton seed per sample. Harvest Oct. Storage at –20 ± 5°C for 20-48 days.

[2] RP-95017, Gaydosh 2000e, GLP, low volume spray, three applications before and three after boll opening. Spray volume including oil 112-140 l/ha for each application, using a back pack CO_2 sprayer (MS) or a tractor-operated boom sprayer (TX). No unusual weather conditions (Jun-Oct, temp 11-39°C; total rain 35-43 cm). USDA soil type sandy loam (TX) or silt loam (MS). Plot size 424-1505 m^2, 2 field samples/plot; kg/sample not stated. Harvest Sept/Oct. Storage at < –20°C for 327-376 days.

Straw, fodder and forage of cereal grains and grasses (group 051)

Results of trials on range grass in the USA in 1991 and 1992 are shown in Table 130.

Samples were analysed for diflubenzuron by HPLC method RES020. Residues were corrected for concurrent method recoveries, if <100%, in the 1991 trials (77%-114%) but not in the 1992 trials (79%-97%) or matrix interferences (max 0.22 mg/kg diflubenzuron in fresh grass, <0.05 mg/kg diflubenzuron in dry grass). Because of matrix interferences results below 0.7 mg/kg in fresh grass are not considered valid.

Samples from Camp Crook (WP 250 formulation, 1991 trials) and Hewlett (SC 240 formulation, 1992 trials) were probably mislabelled as residues from the 0.035 kg ai/ha treatment were lower than from the 0.018 kg ai/ha treatment, both for fresh and dry grass.

Table 130. Residues of diflubenzuron in range grass, USA.

Location, year, variety	Form.	No.	kg ai/ha	kg ai/hl	PHI, days	Sample	diflubenzuron, mg/kg	Ref
Low volume ground spray								
Germansville, (PA), 1991, Kentucky 31	SC 240	1	0.018	0.043	0	fresh grass	1.9, 2.1; mean 2.0	1
LaBelle, (FL), 1991, Argentine Bahia	SC 240	1	0.018	0.039	0	fresh grass	1.6, 3.0; mean 2.3	1
Eakly, (OK), 1991, Common Bermuda	SC 240	1	0.018	0.040	0	fresh grass	0.59, 0.71; mean 0.65	1
Hickman, (CA), 1991, Bermuda	SC 240	1	0.018	0.039	0	fresh grass	2.3, 3.2; mean 2.7	1
Germansville, (PA), 1991, Kentucky 31	WP 250	1	0.018	0.043	0	fresh grass	1.6, 2.3; mean 2.0	1
LaBelle, (FL), 1991, Argentine Bahia	WP 250	1	0.018	0.039	0	fresh grass	2.9, 3.8; mean 3.4	1
Eakly, (OK), 1991, Common Bermuda	WP 250	1	0.018	0.039	0	fresh grass	1.5 (2); mean 1.5	1
Hickman, (CA), 1991, Bermuda	WP 250	1	0.018	0.039	0	fresh grass	1.6, 2.0; mean 1.8	1
Germansville, (PA), 1991, Kentucky 31	SC 240	1	0.035	0.085	0	fresh grass	3.2, 3.5; mean 3.4	1
LaBelle, (FL), 1991, Argentine Bahia	SC 240	1	0.035	0.075	0	fresh grass	2.8, 4.2; mean 3.5	1
Eakly, (OK), 1991, Common Bermuda	SC 240	1	0.035	0.078	0	fresh grass	1.1, 1.3; mean 1.2	1
Hickman, (CA), 1991, Bermuda	SC 240	1	0.035	0.075	0	fresh grass	3.0, 3.8; mean 3.4	1
Germansville, (PA), 1991, Kentucky 31	WP 250	1	0.035	0.084	0	fresh grass	2.7, 2.9; mean 2.8	1
LaBelle, (FL), 1991, Argentine Bahia	WP 250	1	0.035	0.075	0	fresh grass	2.9, 5.5; mean 4.2	1
(Eakly, OK), 1991, OK Common Bermuda	WP 250	1	0.035	0.075	0	fresh grass	2.3, 2.6; mean 2.5	1
Hickman, (CA), 1991, Bermuda	WP 250	1	0.035	0.075	0	fresh grass	2.4, 2.8; mean 2.6	1

Location, year, variety	Form.	No.	kg ai/ha	kg ai/hl	PHI, days	Sample	diflubenzuron, mg/kg	Ref
ULV aerial spray								
Camp Crook, (SD), 1991, -	SC 240	1	0.018	0.78	1	fresh grass dry grass	0.59, 0.61; mean 0.60 0.24, 0.31; mean 0.28	1
Ekalaka, (MT), 1991, -	SC 240	1	0.018	0.78	1	fresh grass dry grass	0.75, 1.0; mean 0.88 0.86 (2); mean 0.86	1
York, (NE), 1992, Brome Grass	SC 240	1	0.018	0.78	0	fresh grass dry grass	0.66, 0.73; mean 0.70 1.9, 2.3; mean 2.1	2
Hettinger, (ND), 1992, Crested Wheat	SC 240	1	0.018	0.78	0	fresh grass dry grass	2.0, 2.4; mean 2.2 2.1, 2.2; mean 2.2	2
Hewlett, (WY), 1992, Crested and Western Wheat	SC 240	1	0.018	0.78	0	fresh grass dry grass	2.4, 2.5; mean 2.4 3.2, 3.6; mean 3.4	2
Camp Crook, (SD), 1991, -	WP 250	1	0.018	0.78	1	fresh grass dry grass	4.6, 4.7; mean 4.6 4.5, 5.7; mean 5.1	1
Ekalaka, (MT), 1991, -	WP 250	1	0.018	0.78	1	fresh grass dry grass	0.63, 1.0; mean 0.84 0.41, 0.81; mean 0.61	1
York, (NE), 1992, Brome Grass	WP 250	1	0.018	0.78	0	fresh grass dry grass	0.33, 0.41; mean 0.37 1.1, 1.2; mean 1.1	2
Hettinger, (ND), 1992, Crested Wheat	WP 250	1	0.018	0.78	0	fresh grass dry grass	2.2, 2.9; mean 2.6 2.6, 2.9; mean 2.7	2
Hewlett, (WY), 1992, Crested and Western Wheat	WP 250	1	0.018	0.78	0	fresh grass dry grass	0.89, 0.96; mean 0.92 1.1, 1.2; mean 1.2	2
Camp Crook, (SD), 1991, -	SC 240	1	0.035	1.5	1	fresh grass dry grass	2.7, 3.0; mean 2.9 3.1, 3.5; mean 3.3	1
Ekalaka, (MT), 1991, -	SC 240	1	0.035	1.5	1	fresh grass dry grass	0.95, 1.0; mean 1.0 0.97, 1.2; mean 1.1	1
York, (NE), 1992, Brome Grass	SC 240	1	0.035	1.5	0	fresh grass dry grass	0.72, 1.1; mean 0.92 2.5, 2.6; mean 2.5	2
Hettinger, (ND), 1992, Crested Wheat	SC 240	1	0.035	1.5	0	fresh grass dry grass	4.4, 4.6; mean 4.5 2.8, 3.7; mean 3.3	2
Hewlett, (WY), 1992, Crested and Western Wheat	SC 240	1	0.035	1.5	0	fresh grass dry grass	0.50, 0.84; mean 0.67 1.2, 1.6; mean 1.4	2
Camp Crook, (SD), 1991, -	WP 250	1	0.035	1.5	1	fresh grass dry grass	0.25, 0.28; mean 0.26 0.63, 1.1; mean 0.86	1
Ekalaka, (MT), 1991, -	WP 250	1	0.035	1.5	1	fresh grass dry grass	1.1, 2.1; mean 1.6 1.3, 1.5; mean 1.4	1
York, (NE), 1992, Brome Grass	WP 250	1	0.035	1.5	0	fresh grass dry grass	0.60, 0.74; mean 0.67 1.8, 1.9; mean 1.9	2
Hettinger, (ND), 1992, Crested Wheat	WP 250	1	0.035	1.5	0	fresh grass dry grass	4.3, 4.7; mean 4.5 4.3, 5.1; mean 4.7	2
Hewlett, (WY), 1992, Crested and Western Wheat	WP 250	1	0.035	1.5	0	fresh grass dry grass	3.3, 4.3; mean 3.8 2.0, 4.4; mean 3.2	2

[1] RP-91053, Gaydosh, 1994b, GLP, spray volume 41-47 l/ha in water for low volume spray and 2.3 l/ha (in 100% diesel oil or water/diesel oil (80:20)) for ultra low volume aerial spray. Weather conditions (Sept-Dec) and soil not stated. Above-ground leaves and stems of grass were handpicked (plot size not stated; 2 field samples/plot; 1.4 kg grass/sample). Fresh grass was immediately stored frozen, while dried grass was laid out to dry. Harvest Sept-Dec. Storage at –15 ± 5°C for 415-547 days.

[2] RP-92001, Gaydosh, 1993b, GLP, spray volume 2.3 l/ha (in 100% diesel oil) for ultra low volume aerial spray. No unusual weather conditions (Jun, Aug, Sept, temp 6-30°C, 0 cm rain). USDA soil type sandy loam (Hewlett, WY; pH 8.1), loamy sand (Hettinger, ND; pH 7.8) or clay loam (York, NE, pH 6.4). Above-ground leaves and stems of grass were handpicked (plot size >2 ha; 2 field samples/plot; 1.4 kg grass/sample). Fresh grass was immediately stored frozen, while dried grass was laid out to dry: one day in Hettinger (ND) and Hewlett (WY), and 2.5 days in York (NE). Samples were cut into small segments and sub-sampled. Harvest Jun/Aug/Sept. Storage at –20 ± 5°C for 160-294 days with one day at –12.6°C.

FATE OF RESIDUES IN STORAGE AND PROCESSING

In storage

The Meeting received information on the fate of incurred residues of diflubenzuron during the storage of wheat grain.

Residue data from a wheat storage trial in Australia are shown in Table 131 (Heekin and Rich, 1987, non-GLP). Two 5-tonne batches of wheat grain were treated with a single post-harvest spray (formulation and spray concentration not reported) at 2 and 4 g ai/tonne respectively. The wheat was kept at ambient temperature for 9 months (storage conditions not reported). Laboratory samples were stored at –20°C until analysis (storage time not reported) and were analysed by the Australian HPLC method for diflubenzuron. Results were not corrected for concurrent method recoveries (93-98%) or matrix interferences (no peaks detected). Variability within laboratory subsamples was 1.4%-1.8% for the 2 g ai/tonne treatment and 3.4%-4.7% for the 5 g ai/tonne treatment.

Table 131. Residues of diflubenzuron during storage of post-harvest treated wheat grain.[1]

Location, year, variety	DAT (months)	diflubenzuron, mg/kg 1x 2 g ai/tonne	diflubenzuron, mg/kg, 1x 4 g ai/tonne
Australia, Bangalla, year ?, -	0	1.8^2	3.5^3
	1	1.5^2	4.4^3
	2	1.5^2	3.6^3
	3	1.5^2	3.7^3
	4	1.6^2	3.3^2
	5	1.4^3	3.3^3
	6	1.4^3	3.8^3
	7	1.5^3	3.9^3
	9	1.6^3	3.2^3

[1] DI-5937, Heekin and Rich, 1976, non-GLP. Results mean of 2 or 3 laboratory sub-samples, as indicated by superscripts.

In processing

The Meeting received information on the fate of residues of diflubenzuron during the processing of oranges, apples, pears, plums, mushrooms, rice, wheat and soya bean. Processing factors (P-factors) were calculated as the ratio (residue in processed commodity): (residue in raw agricultural commodity) (RAC). Percentage transfer (%T) was calculated as P-factor x (weight of processed commodity ÷ weight of RAC).

Orange oil. Oranges (Hamlin round) were topically treated twice in a greenhouse (Lake Alfred, FL, USA, 1989) with acetone solutions of diflubenzuron in which both the phenyl rings were labelled with ^{14}C, at rates equivalent to 0.35 kg ai/ha at an interval of 14 days. Fruits were harvested 21 days after the second application. Citrus oil was made from two samples consisting of 88 and 79 oranges, in small portions of 5 oranges. Fruits were washed for 10 min in a detergent solution (0.03% sodium dioctylsulfosuccinate) and dried in air. The oranges were thinly peeled and the peel homogenized in water (25 seconds). The slurry was centrifuged (10 min, 200 g) and the top emulsion layer centrifuged for 10 min at 1900 g. The second emulsion layer was isolated and aged for 1-3 days at room temperature. The aged emulsion was centrifuged for 10 min at 17000 g, when the top layer contained the oil; the remaining emulsion was centrifuged again until no more oil could be separated (Nigg, 1989; Joustra *et al.*, 1989).

For each sample the oil fractions were combined, diluted with n-hexane and extracted four times with ACN. The radioactivity in the unextracted oil was determined by LSC and the compounds in the extract were characterized by HPLC (RP-8 column) with LSC and UV detection.

5 oranges (760 g) yielded 115 g peel, from which 1.1 g pure oil could be separated. 19.4 g citrus oil was isolated from the 88-orange sample, and 17.7 g from the 79-orange sample.

95%-97% of the total ^{14}C present in the oil was extracted. Recoveries from oil spiked before ACN extraction with a mixture of diflubenzuron (0.01 mg/kg), CPU (0.01 mg/kg), PCA (0.02 mg/kg) and DFBA (0.1 mg/kg), were 108% for diflubenzuron, 89% for CPU, 90% for DFBA and 57% for PCA. The results were not corrected for these recoveries.

The whole oranges contained on average a total of 0.66 mg/kg diflubenzuron equivalents of which 95% was the parent (0.63 mg/kg), while CPU, DFBA and PCA were not found and 5% was bound. Citrus oil contained on average 43 mg/kg parent, with no CPU, DFBA or PCA. The processing factor for diflubenzuron in the preparation of oil was 43/0.63 = 68; the percentage transfer was 68x1.1/760 = 9.8%.

Apple sauce, apple juice, apple pomace (wet and dry) and apple butter. Apples were treated in the USA (1983) with a WP250 at 3 or 4 x 0.21 kg ai/ha or 3 x 0.42 kg ai/ha up to 28 days before harvest (Table 132). The apples were processed into canned sauce, pasteurised juice, wet and dry pomace and butter (ABC, 1984, non-GLP). The weights of the RAC and processed commodities were not reported.

Apple sauce. Apples were peeled, cored, cut into pieces and steam-cooked for 3 min (98.8°C) in a thermo-screw cooker. The sauce was collected and, after addition of water and sugar, heated to 68.3-71.0°C and filled into cans at 90.5-96.0°C. The cans were closed and cooled in cold water.

Pomace and apple juice. Apples were ground in a hammer mill, pressed at 35 kg/cm^2 for 5 min, then at 70 kg/cm^2 for 4 min. The resulting juice was pasteurised in at heat-exchanger at 87.7°C, filled into bottles and cooled slowly. The remaining pomace was re-ground, packed in airtight containers, and frozen to –23°C. Some of the wet pomace was spread on trays and dried in a hot-air dryer (76.7°C) for 45-60 min. The resulting dry pomace was cooled and packed in airtight containers. The authors did not state whether the initial apples were washed before grinding.

Apple butter. Apples were crushed in a mill, mixed with 30% water, heated for 10 min at 1.4 kg/cm^2 in a steam-jacketed kettle. After addition of 20% sucrose, the apple mass was similarly heated for 40 min at the same pressure. Jars were filled with butter at 87.7°C, sealed and cooled. The authors did not state whether the apples were washed and peeled before crushing.

Samples were analysed by method RES010 for diflubenzuron. Residues were corrected for concurrent method recoveries (74%-136%), but not for matrix interferences (<0.05 mg/kg diflubenzuron in apples, apple juice and apple sauce; <0.5 mg/kg in wet and dry apple pomace and apple butter) (Table 52). The results are shown in Table 132.

Apples grown in Germany (1993) were treated with four applications of a WP250 or WG800 formulation (1 x 0.30 plus 3 x 0.18 kg ai/ha) up to 28 days before harvest (Thus and Allan, 1995). Field samples were 10-10.5 kg. 12 apples (about 2 kg) were processed into raw juice and wet pomace with a citrus press. The ratio of processed commodity to RAC was about 0.5 for both pomace and juice. Further details of processing were not available.

Samples were analysed for diflubenzuron by HPLC method RES060. Residues were not corrected for concurrent method recoveries (67-108%) or matrix interferences (max. 0.004 mg/kg), see Table 58. The results are shown in Table 132.

Table 132. Residues of diflubenzuron in apples and processed products.

Location, year, variety	Form	kg ai/ha and interval, i	kg ai/hl	DAT	Sample	Diflu-benzuron, mg/kg	P-factor	% T	Ref
USA, Watsonville, CA, 1983, Red Delicious	WP 250	3x0.21; i = 56, 47days	ns	28	whole fruit canned sauce pasteurised juice wet pomace dry pomace apple butter	0.18 <0.05 <0.05 1.1 3.0 <0.5	- <0.28 <0.28 6.1 17 <2.8	wna	1

Location, year, variety	Form	kg ai/ha and interval, i	kg ai/hl	DAT	Sample	Diflu-benzuron, mg/kg	P-factor	% T	Ref
USA, Watsonville, CA, 1983, Red Delicious	WP 250	3x0.42; i = 56, 47days	ns	28	whole fruit	0.86	-	wna	1
					canned sauce	<0.05	<0.058		
					pasteurised juice	<0.05	<0.058		
					wet pomace	3.1	3.6		
					dry pomace	8.0	9.3		
					apple butter	<0.5	<0.58		
USA, Watsonville, CA, 1983, Newton	WP 250	3x0.21; i = 56, 47days	ns	28	whole fruit	0.31	-	wna	1
					canned sauce	<0.05	<0.16		
					pasteurised juice	<0.05	<0.16		
					wet pomace	1.6	5.2		
					dry pomace	4.3	14		
					apple butter	<0.5	<1.6		
USA, Watsonville, CA, 1983, Newton	WP 250	3x0.42; i = 56, 47days	ns	28	whole fruit	0.51	-	wna	1
					canned sauce	<0.05	<0.098		
					pasteurised juice	<0.05	<0.098		
					wet pomace	2.4	4.7		
					dry pomace	6.2	12		
					apple butter	<0.5	<0.98		
USA, Hood River, OR, 1983, Red Delicious	WP 250	3x0.21; i = 33, 63days	ns	28	whole fruit	0.34	-	wna	1
					canned sauce	<0.05	<0.15		
					pasteurised juice	<0.05	<0.15		
					wet pomace	1.9	5.6		
					dry pomace	4.9	14		
					apple butter	<0.5	<1.5		
USA, Hood River, OR, 1983, Red Delicious	WP 250	3x0.42; i = 33, 63days	ns	28	whole fruit	0.64	-	wna	1
					canned sauce	<0.05	<0.078		
					pasteurised juice	<0.05	<0.078		
					wet pomace	3.0	4.7		
					dry pomace	8.6	13		
					apple butter	<0.5	<0.78		
USA, Wapato, WA, 1983, Red Delicious	WP 250	3x0.42; i = 29, 63days	ns	28	whole fruit	0.37	-	wna	1
					canned sauce	<0.05	<0.14		
					pasteurised juice	<0.05	<0.14		
					wet pomace	2.2	5.9		
					dry pomace	6.1	16		
					apple butter	<0.5	<1.4		
USA, Hood River, (OR), 1983, Red Delicious	WP 250	4x0.21; i = 29, 26, 27days	ns	29	whole fruit	0.81	-	wna	1
					canned sauce	<0.05	<0.062		
					pasteurised juice	<0.05	<0.062		
					wet pomace	3.7	4.6		
					dry pomace	9.4	12		
					apple butter	<0.5	<0.62		
Germany, Kirchheim 1993, Jonagold	WP 250	1x0.30; 3x0.18; i = 32, 33, 31days	1x0.060 3x0.036	28	whole fruit	0.12	-	-	2
					raw juice	0.017	0.14	7.0	
					wet pomace	0.25	2.1	105	
Germany, Ersdorf 1993, James Grieve	WP 250	1x0.30; 3x0.18; i = 20, 18, 20days	1x0.020 3x0.012	28	whole fruit	0.29	-	-	2
					raw juice	0.025	0.086	4.3	
					wet pomace	0.37	1.3	65	
Germany, Kirchheim 1993, Jonagold	WG 800	1x0.30; 3x0.18; i = 32, 33, 31days	1x0.060 3x0.036	28	whole fruit	0.11	-	-	2
					raw juice	0.016	0.15	7.5	
					wet pomace	0.26	2.4	120	
Germany, Ersdorf 1993, James Grieve	WG 800	1x0.30; 3x0.18; i = 20, 18, 20days	1x0.020 3x0.012	28	whole fruit	0.36	-	-	2
					raw juice	0.022	0.061	3.0	
					wet pomace	0.39	1.1	55	

wna: weights of RAC and processed commodities not available

ns: not stated

[1] DI-4693, ABC, 1984, non-GLP, storage frozen for 253-266 days (temperature not stated). Further details in Table 118, footnote 23.

[2] DI-9320, Thus and Allan, 1995, non-GLP, storage 180-262 days at –10°C. Further details in Table 118, footnote 27.

Canned pears. Pears were treated in the USA (1983) with three applications of a WP250 formulation at 0.21 or 0.42 kg ai/ha up to 28 days before harvest and canned (ABC, 1984, non-GLP). Details were not available. The weights of the RAC and processed commodity were not reported.

Samples were analysed for diflubenzuron by method RES010. Residues were corrected for concurrent method recoveries (80%-100%), but not for matrix interferences (<0.05 mg/kg diflubenzuron) (Table 52). Results are shown in Table 133.

Table 133. Residues of diflubenzuron in pears and canned pears, USA, 1983.[1]

Location	Form	kg ai/ha	kg ai/hl	DAT	Sample	diflubenzuron mg/kg	P-factor
Wapato, WA	WP 250	3x 0.21 i=29,35	3x 0.006	27	whole fruit canned pears	0.29 <0.05	- <0.17
Hood River, OR	WP 250	3x 0.21 i=33,30	3x 0.022	28	whole fruit canned pears	0.22 <0.05	- <0.23
Wapato, WA	WP 250	3x 0.42 i=29,35	3x 0.011	27	whole fruit canned pears	0.53 <0.05	- <0.094
Hood River, OR	WP 250	3x 0.42 i=33,30	3x 0.045	28	whole fruit canned pears	0.54 <0.05	- <0.093

[1] DI-4693, ABC, 1984, non-GLP, storage frozen 293-314 days (temperature not stated). Further details in Table 119, footnote 14.

Prunes. Plums were treated twice in the USA (Woodland, CA, 1998, variety French prunes) with a WP 250 at 2.2 plus 1.1 kg ai/ha. Further details are given with the corresponding supervised residue trials (Gaydosh 2000b, Table 121). Mature fruits were washed and dried for 18 hours. Fresh fruit yielded 31-33% of prunes.

The total storage time from harvest to last analysis was 127-200 days for diflubenzuron and 231-298 days for PCA. The samples were analysed by GC-ECD method LAI 3-86-6 for diflubenzuron and GC-ID-MS method I for PCA; CPU was not determined. Residues were corrected for concurrent recoveries if <100% (69%-72% for diflubenzuron and 98-99% for PCA), but not for matrix interferences (<0.015 mg/kg for diflubenzuron and <0.0015 mg/kg for PCA) (Table 74 for diflubenzuron and Table 93 for PCA).

The RAC contained 0.15 and 0.21 (mean 0.18) mg/kg diflubenzuron and the prunes 0.16 and 0.20 (mean also 0.18). PCA was below the LOQ of 0.005 mg/kg in both plums and prunes. The processing factor is therefore 0.18/0.18 = 1 and the percentage transfer 32%.

Canned mushrooms. Four boxes containing compost were spawned with *Agaricus bitorquis*, two with a compost layer of 17 cm, the other two with a layer of 10 cm. Diflubenzuron in which the difluorobenzoyl moiety was labelled with ^3H or the chloroaniline moiety with ^{14}C was added as a dispersion in water at a dose of 10 kg ai/ha to the compost on day 1. At casing (day 13) the compost was covered with a 4 cm layer of casing soil and each box received another dose of 10 kg ai/ha. Four flushes of mushrooms were harvested on days 32, 38, 46 and 55. Mushrooms from each flush and from each box were considered to be one sample. 50 g of homogenized mushrooms plus 25 ml of blanching solution (1% NaCl and 0.1% citric acid) were heated to 95-100°C and the mixture autoclaved at 116°C for 20 or 40 min. After flash-cooling to room temperature, the canned mushrooms were extracted twice with ACN and twice with methanol/water (1:1) and the extracts pooled (Nimmo and De Wilde, 1977).

The extracts were analysed by LSC and the unextractable residues by combustion and LSC. Diflubenzuron, CPU, PCA and DFBA were determined by reversed isotope-dilution analysis. The identity of the metabolites was confirmed by TLC (silica gel; two solvent systems) with autoradiographic and UV detection.

The results were not shown, only the conclusions were stated by the authors. In unprocessed mushrooms, the main metabolite with the ^{3}H label was DFBA, and the main metabolite with the ^{14}C label was CPU. Processing homogenized mushrooms at 116°C did not influence the DFBA residue but lowered that of CPU by approximately 30%, after both 20 and 40 min sterilization. The expected breakdown product PCA was not found.

Compost was spawned with *Agaricus bisporus*, var. U1 and diflubenzuron ^{14}C-labelled in both phenyl rings added as a suspension to duplicate compost and casing samples at a dose of 50 kg ai/ha. Mushrooms from three flushes were combined and two samples of 100 g were used for canning. Mushrooms from each sample were kept in 500 ml of evacuation solution (0.1% citric acid and 0.05% Na_2SO_3) for 15 min under reduced pressure, transferred to 100 ml of demi-water, blanched for 7 min and transferred to water at a temperature of 2-10ºC. The mushrooms were transferred to 100 ml of sterilization solution (0.1% citric acid and 1% NaCl), sterilized for 25 min at 116ºC and cooled. All solutions from one sample were pooled. Canned and uncanned mushrooms were extracted successively with 0.5 M HCl/ACN (1:1) and ACN and the extracts pooled. Part of the pooled extract was extracted with diethyl ether, the remaining layer made alkaline (pH 9) and extracted again with diethyl ether, and the ether extracts pooled. Extracts and pooled solutions were analysed by LSC or HPLC/LSC and unextractable residues by combustion and LSC (Thus and Van der Laan-Straathof, 1993b).

During canning more than 70% of the radioactivity in the mushrooms moved into the canning liquid. The total radioactive residue in canned mushrooms was 0.05-0.09 mg/kg (mean 0.07) as diflubenzuron from the compost treatment and 0.85-1.0 mg/kg (mean 0.94) from the casing treatment. The composition of the ^{14}C residues in uncanned and canned mushrooms and in the canning liquid in presented in Table 134. The main part of the residue in the canning liquid consisted of DFBA (>100%) and in the canned mushrooms consisted of the parent (1.8%-14%), DFBA (46%-72%) and bound residues (20%-39%).

Table 134. Mean composition of ^{14}C residues in raw and canned mushrooms and in the canning liquid.

	Compost treatment				Casing treatment			
	RAC mg/kg	liquid mg/kg[a]	canned mushrooms		RAC mg/kg	liquid mg/kg[a]	canned mushrooms	
			mg/kg[a]	% of TRR			mg/kg[a]	% of TRR
Total ^{14}C	0.21	0.19	0.070	100	8.0	5.9	0.94	100
parent[1]	0.004	0.040	0.010	14	0.04	0.039	0.017	1.8
DFBA[1,3]	0.17	0.24	0.032	46	7.1	6.0	0.68	72
CPU[2]	0.0008	0.040	0.0004	0.6	0.06	0.12	0.008	0.9
PCA[2,3]	0.002	0.040	0.0009	1.3	0.05	0.11	0.018	2.0
OH-DFB[1]	0.01		0.0004	0.6	0.02		0.0007	0.1
bound	0.02		0.028	39	0.27		0.19	20
P-factor (diflubenzuron)	-		2.5		-		0.43	
%T (diflubenzuron)	-		wna				wna	

wna: weights of RAC and processed commodities not available

[a] concentration expressed as mg/kg diflubenzuron equivalents

[1] ACN extracts

[2] diethyl ether extracts

[3] results were corrected for recovery: DFBA 82% and PCA 24%

Soya bean oil. In a semi-open greenhouse, leaves and developing pods of soya bean plants (Glycine Max, var. Evasoy) were treated once or twice at an interval of 3 weeks with diflubenzuron (WP formulation) in which both phenyl rings were labelled with ^{14}C (specific activity 19.6 mCi/g; radiochemical purity 99.5%). The dosage rate was equivalent to 0.07 kg ai/ha. The radiolabelled suspension was applied spotwise using a syringe. Pods were sampled at days 18 and 62 (at harvest). Mature pods were separated into seeds and hulls (Thus and Van der Laan-Straathof, 1993a).

Samples were extracted within 24 hours. Homogenized plant parts were extracted successively with ACN (twice) and methanol, and the extracts pooled. For oil extraction, homogenized seeds were Soxhlet-extracted for 4 h with hexane and the hexane was evaporated, leaving the oil. Extracts, oil and unextractable residues were counted by LSC, and extracts characterized by HPLC (C-18 column) with LSC and UV detection. Reference compounds were diflubenzuron, CPU, DFBA and PCA.

Of the total the applied radioactivity, 89%-105% was recovered: >99% of the recovered radioactivity was found in the treated leaves and pods and less than 0.2% in the untreated parts. In the treated pods, >99% of the recovered radioactivity was in the hulls and less than 0.2% in the seeds. In the oil ^{14}C residues were near the LOQ (0.01 mg/kg) and there was no significant difference between oil from treated and untreated pods: 0.014 and <0.01-0.012 mg/kg respectively.

Polished rice, rice hulls and bran. Rice was treated in the USA (1995, 1996) with an SC240 or WP250 formulation as a single low volume ground or aerial spray at 2.2 kg ai/ha. Further details are given with the corresponding supervised residue trials (Table 127, footnotes 4 and 5)

In the 1995 trials a stationary thresher was used to separate grain and straw. Commercial (small scale) processing of the samples consisted of drying to a moisture level between 11 and 14% in an oven, aspiration to remove light impurities, and screen cleaning. The cleaned rice was passed through a dehuller to give hulls and brown rice and the brown rice was decorticated in a mill to produce polished rice and bran. Whole rice yielded 16%-19% hulls, 9%-14% bran and 63%-67% polished rice.

Processing in the 1996 trials was similar except that the rough rice was dried to a moisture level of 12% in a heated, forced-air sample dryer. Whole rice yielded 15%-20% hulls, 11%-23% bran and 47%-59% polished rice.

The samples were analysed by GC-ECD method LAI 3-86-6 for diflubenzuron, GC-ECD method LAI 3-86-9 for CPU and GC-ID-MS method PTRL 645W for PCA. Residues were not corrected for concurrent recoveries (58%-123% for diflubenzuron, 65%-142% for CPU, 60%-112% for PCA) or matrix interferences (below the reported LOQ) (Table 74 diflubenzuron; Table 83 CPU, and Table 93 PCA). Storage stabilities of diflubenzuron and CPU was not validated beyond 1 month, during which they were stable. Residues in all except the 1996 MS trial were too low to be useful. The results are shown in Table 135.

Table 135. Residues of diflubenzuron and metabolites in rice treated at 2.2 kg ai/ha and its processed fractions, USA.

Location, year	Sample	diflubenzuron (mg/kg)	CPU (mg/kg)	PCA (mg/kg)	Difluben-zuron equivalents mg/kg^1	P-factor	% T	P-factor^1	% T^1	Ref.
MS, 1996	whole rice	0.26, 0.87; mean 0.56	0.006; 0.012; mean 0.009	<0.005, 0.005; mean 0.005	0.59	-	-	-	-	^{2}
	rice hull	0.21, 0.23; mean 0.22	<0.005 (2); mean <0.005	0.009; 0.010; mean 0.010	0.25	0.39	0.08	0.42	0.08	
	rice bran	0.05, 0.11; mean 0.08	<0.005 (2); mean <0.005	<0.005 (2); mean <0.005	0.10	0.14	0.03	0.17	0.04	

Location, year	Sample	diflubenzuron (mg/kg)	CPU (mg/kg)	PCA (mg/kg)	Difluben-zuron equivalents mg/kg[1]	P-factor	% T	P-factor[1]	% T[1]	Ref.
	polished rice	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005	<0.024	<0.018	<0.01	<0.041	<0.01	
TX, 1996	whole rice	<0.01 (2); mean <0.01	0.006, 0.008; mean 0.007	<0.005 (2); mean <0.005		na	na	na	na	[2]
	rice hull	<0.05 (2); mean <0.05	0.007, 0.012; mean 0.010	0.011 (2); mean 0.011		na	na	na	na	
	rice bran	0.01 (2); mean 0.01	0.025, 0.033; mean 0.029	<0.005; 0.006; mean 0.006		na	na	na	na	
	polished rice	<0.01 (2); mean <0.01	0.002 (2); mean 0.002	<0.005 (2); mean <0.005		na	na	na	na	
CA, 1995	whole rice	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005		na	na	na	na	[3]
	rice hull	<0.05 (2); mean <0.05	<0.005 (2); mean <0.005	<0.005 (2); mean <0.005		na	na	na	na	
	rice bran	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005	<0.005 (2); mean <0.005		na	na	na	na	
	polished rice	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005		na	na	na	na	
MS, 1995	whole rice	<0.01, 0.02; mean 0.02	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005		na	na	na	na	[3]
	rice hull	<0.05 (2); mean <0.05	<0.005 (2); mean <0.005	<0.005 (2); mean <0.005		na	na	na	na	
	rice bran	<0.01 (2); mean <0.01	<0.005 (2); mean <0.005	<0.005 (2); mean <0.005		na	na	na	na	
	polished rice	<0.01 (2); mean <0.01	<0.001 (2); mean <0.001	<0.005 (2); mean <0.005		na	na	na	na	

na: not applicable, residues at or near LOQ

[1] Diflubenzuron equivalents = mg/kg diflubenzuron plus 1.821 x mg/kg CPU plus 2.435 x mg/kg PCA

[2] Gaydosh *et al.*, 1997b, GLP. Storage at <-20°C 351-388 days for diflubenzuron, 499-542 days for CPU and 563-613 days for PCA. Further details in Table 127, footnote 4.

[3] Gaydosh, 1998b, GLP. Storage at <-20°C for 178-214 days for diflubenzuron, 217-237 days for CPU and 222-245 days for PCA. Further details in Table 127, footnote 5.

<u>Milling and baking fractions of wheat</u>. Heekin and Rich (1987, non-GLP) spray-treated a 5-tonne batch of wheat grain post-harvest with diflubenzuron (formulation not reported) at 2 g ai/tonne and kept the wheat at ambient temperature for 4 months (storage conditions not reported). Samples were taken from the surface and at 1 and 2 m depths. Sub-samples (6 kg each) were sieved and water was added to bring the moisture content up to 15%. Samples were processed into Buehler flour, bran and offals, first reduction flour (40% of Buehler flour), wholemeal flour, white bread (made from Buehler flour) and wholemeal bread (made from wholemeal flour). Processing details were not provided and therefore mass fractions could not be calculated. Samples were stored at –20°C (time not reported). Samples (30 g flour, 15 g bran and offals, 60 g bread) were analysed by the Australian HPLC method. Results were corrected for concurrent method recoveries (93%-107%), but not for matrix interferences (no peaks visible) (Table 54). Diflubenzuron concentrations were expressed on a wet weight basis, but can be calculated on a dry weight basis using the moisture content (overnight drying at 110°C). Because the ring compound 1-*p*-chlorophenyl-5-fluoroquinazoline-2,4(1*H*,3*H*)-dione is expected in bread after loss of HF during baking, the analytical method was modified (different mobile phase) to detect it (concurrent method recoveries at 1 mg/kg (56-83% in wholemeal bread and 91-92% in white bread), but the compound was not found in white or wholemeal bread (reported LOQ 0.03 mg/kg).

Table 136. Residues of diflubenzuron and metabolites in wheat grain treated post-harvest at 2 g ai/tonne and stored at ambient temperatures for 4 months and in processed fractions (Heekin and Rich, 1987, non-GLP).

Location, year	Sample	Moisture (% w/w)	diflubenzuron (mg/kg wet weight)[1]			P-factor
			surf.	1 m	2 m	
Australia, Bangalla, year?, -	wheat grain	11.0	1.8^{2}	1.6^{2}	1.6^{2}	-
	sieved wheat	11.0	1.2^{5}	1.4^{6}	1.4^{6}	0.70; 0.87; 0.87
	bran	13.5	3.4^{2}	3.6^{2}	3.7^{2}	1.9; 2.3; 2.3
	offals	12.6	1.3^{2}	1.2^{2}	1.4^{2}	0.72; 0.79; 0.90
	Buehler flour	13.3	0.54^{2}	0.55^{2}	0.53^{2}	0.15; 0.17; 0.18
	1st reduction	13.3	0.27^{2}	0.27^{2}	0.29^{2}	0.32; 0.35; 0.34
	flour	13.2	1.1^{2}	1.1^{2}	1.2^{2}	0.62; 0.72; 0.74
	wholemeal flour	36.7	0.35^{2}	0.36^{4}	0.34^{4}	0.20; 0.23; 0.22
	white bread	41.4	0.71^{3}	0.76^{4}	0.74^{4}	0.40; 0.49; 0.47
	wholemeal bread					

[1] Results are means of 2, 3, 4, 5 or 6 analytical replicates, indicated by superscripts in the Table

Residues in the edible portion of food commodities

No information available

RESIDUES IN ANIMAL COMMODITIES

Direct animal treatments

Reports were submitted only for sheep, although use patterns exist for cattle, sheep and fish.

Forty male Merino sheep (5-8 years old) were treated in Australia (The Oaks, NSW) with a 25.0 g/l diflubenzuron pour-on formulation against sheep lice (Shepherd, 1998a). The sheep were divided into eight groups of five and then shorn; the post-shearing weight ranged from 34.9 to 50.1 kg. Groups 1-7 were treated within 24 hours after shearing along the dorsal mid-line between the points of the poll and rump at a dose of 20.2-22.6 mg ai/kg bw, corresponding to 30-40 ml product per sheep (1.5-2 times the standard dose). Group 8 remained untreated as controls. Groups of 5 animals was slaughtered 1, 3, 7, 14, 21, 42, and 84 days after treatment. Tissue samples (lumbar, pre-femoral and perirenal fat, muscle, liver and kidney) were collected and stored at –15°C (maximum 92 days), then analysed by HPLC method STM CR 59 for diflubenzuron. Residues were not corrected for concurrent method recoveries (67%-113%) nor for matrix interferences (<0.005 mg/kg for muscle and fat and <0.01 mg/kg for kidney and liver) (Table 70).

Results are shown in Table 137. Residues in the liver, kidney and muscle were below the LOQ (<0.02 mg/kg) in all samples except a single liver sample one day after treatment (0.02 mg/kg). Residues were found at random in fat on days 1 to 21 after treatment (<0.02-0.05 mg/kg), with the greatest persistence in the perirenal and lumbar fat. Diflubenzuron residues were below the LOQ (<0.02 mg/kg) after 21 days in perirenal fat and after 42 days in pre-femoral and lumbar fat.

After 14 days one animal showed anomalous high residues in fat (0.23-0.50 mg/kg) compared with the other animals in that group (0.03 mg/kg maximum). Low residues were also found in the liver (0.03 mg/kg) and muscle (0.02 mg/kg) of this animal.

Table 137. Diflubenzuron residues in ovine tissues after off-shear pour-on treatment.

Waiting period (days)	Sheep no	Lumbar fat	Pre-femoral fat	Perirenal fat	Muscle	Kidney	Liver
1	926	<LOQ	<LOQ	<LOQ	<DL	<DL	<DL
	934	na	0.02	0.03	<DL	<DL	<DL
	938	<LOQ	0.02	0.03	<DL	<DL	0.02
	948	na	<LOQ	<LOQ	<DL	<DL	<DL
	955	na	<LOQ	0.02	<DL	<DL	<DL
3	919	<DL	<LOQ	<LOQ	<DL	<DL	<DL
	921	0.02	0.02	0.02	<DL	<DL	<DL
	932	0.04	<LOQ	<LOQ	<DL	<DL	<DL
	942	<LOQ	0.02	<LOQ	<DL	<DL	<DL
	944	<LOQ	0.04	<LOQ	<DL	<DL	<DL
7	928	<LOQ	<DL	<LOQ	<DL	<DL	<DL
	937	<LOQ	<LOQ	0.02	<DL	<DL	<DL
	943	<LOQ	<LOQ	<LOQ	<DL	<DL	<DL
	953	0.02	<LOQ	<LOQ	<DL	<DL	<DL
	954	0.03	0.03	0.02	<DL	<DL	<DL
14	923	0.03	<DL	<DL	<DL	<DL	<DL
	927	0.02	0.02	<DL	<LOQ	<DL	<DL
	970	0.23; 0.26	0.36	0.50	0.02	<LOQ	0.03
	1010	0.02	<LOQ	<LOQ	<DL	<DL	<DL
	1011	<LOQ	<DL	<LOQ	<DL	<DL	<DL
21	917	<LOQ	<LOQ	<DL	<DL	<DL	<DL
	924	<LOQ	<LOQ	<LOQ	<DL	<DL	<DL
	940	0.03	0.05	<DL	<DL	<DL	<DL
	941	<DL	<LOQ	<DL	<DL	<DL	<DL
	946	<DL	<LOQ	<DL	<DL	<DL	<DL
42	925	<DL	<DL	<DL	<DL	<DL	<DL
	935	<LOQ	<DL	<DL	<DL	<DL	<DL
	951	<DL	<DL	<DL	<DL	<DL	<DL
	952	<DL	<DL	<DL	<DL	<DL	<DL
	1009	<LOQ	<DL	<DL	<DL	<DL	<DL
84	927	<DL	na	na	na	na	na
	931	<DL	na	na	na	na	na
	956	<DL	na	na	na	na	na
	no tag	<DL	na	na	na	na	na
	ns	na	na	na	na	na	na
control	ns	na	na	na	na	na	na

na: not analysed
ns: not specified
DL (detection limit) 0.005 mg/kg for muscle and fat; 0.01 mg/kg for liver and kidney
LOQ 0.02 mg/kg for all tissues

Thirty five merino lambs (22 male, 13 female; 5 months old) were treated at the same site with the same formulation against blowfly (Shepherd, 1998b). The lambs were divided into seven groups of five, each group having similar body weights (16.0-24.0 kg). Groups 1-6 were treated with two 17 ml bands, one on either side of the backline between the base of the neck and the rump, with a further 17 ml applied to the crutch area (51 ml product per animal), corresponding to a rate of 51.0-75.0 mg ai/kg bw. Group 7 remained untreated as controls. Groups of 5 animals were slaughtered 1, 3, 7, 14, 21, and 42 days after treatment. Lumbar, pre-femoral and perirenal fat samples were stored at –15°C (max. 50 days). Other tissues were not analysed because of the results of the previous study. Samples were analysed as before. Residues were not corrected for concurrent method recoveries (77%-82%) or matrix interferences (<0.005 mg/kg).

The results are shown in Table 138. Residues were found randomly in fat samples from 1-21 days (<0.02-0.13 mg/kg), and after 42 days in one pre-femoral and one lumbar sample from different animals (0.04 mg/kg).

Table 138. Diflubenzuron residues in ovine tissues after spray-on treatment.

Waiting period (days)	Sheep no	Lumbar fat	Pre-femoral fat	Perirenal fat
1	1761	<LOQ	<LOQ	0.02
	1764	<DL	<DL	<DL
	1770	<DL	<DL	<DL
	1777	<DL	<DL	<DL
	1780	0.05	<DL	<DL
3	1760	0.03	0.02	0.03
	1775	<LOQ	<DL	<LOQ
	1792	<DL	<LOQ	<LOQ
	1797	0.09	<DL	<LOQ
	1800	<DL	0.06	0.03
7	1763	<LOQ	<LOQ	<LOQ
	1767	0.05	<LOQ	<LOQ
	1779	0.05	<LOQ	<LOQ
	1782	0.07	<DL	0.02
	1783	0.13	0.08; 0.04	<DL
14	1765	0.04	<DL	<LOQ
	1772	0.03	<LOQ	0.02
	1781	0.07	<LOQ	<LOQ
	1794	0.03	<LOQ	0.02
	1795	<LOQ	<LOQ	<LOQ
21	1761	<DL	<DL	<LOQ
	1762	<DL	0.13; <DL	<DL
	1778	<LOQ	<LOQ	<LOQ
	1786	<DL	<DL	<LOQ
	1796	<DL	<DL	<LOQ
42	1771	<LOQ	<DL	<DL
	1776	<LOQ	0.04	<DL
	1789	<LOQ	<DL	<DL
	1793	0.04	<DL	<LOQ
	1798	<DL	<DL	<DL
control	ns	na	na	na

na: not analysed
ns: not specified
DL (detection limit) 0.005 mg/kg
LOQ 0.02 mg/kg

Six Romney ewes were treated in New Zealand (Hamilton) with Fleececare (SC 250) by plunge-dipping (Southworth, 1991). Each ewe was plunged individually for 3 min in the solution (1.5 L product in 1000 l water) to give total saturation of the fleece. The six sheep retained on average 4 l of dip solution, corresponding to 1.5 g ai/sheep. Some swallowing of dip wash may have occurred. Three sheep were slaughtered after 15 hours and three after 7 days. Liver, kidney and muscle were collected and stored frozen (temperature and duration not reported). Samples were analysed in duplicate by HPLC method C for diflubenzuron. Residues were not corrected for concurrent method recoveries. There were no control samples.

No residues were found in liver or kidney (<0.03 mg/kg); the muscle samples were not analysed because of the absence of residues in liver and kidney. The author did not state why no fat or milk samples were taken.

The first 34 pages of the study report were not submitted.

Twenty Texel cross Shetland sheep less than 1 year old were treated in the UK (Harrogate, North Yorkshire) with a 24.4 g/l diflubenzuron pour-on formulation (Batten, 2000). The sheep were shorn 7 days before treatment and the fleece length was 0.8-2.0 cm (mainly 1.0-1.5 cm) on the day of dosing. The sheep were divided into five groups of four (2 male and 2 female), each group having similar body weights (36-45 kg at dosing). Groups 1-4 were treated with two 17 ml bands along either side of the spine and a further 17 ml around the crutch (51 ml product per animal). The dose rate corresponds to 28-35 mg ai/kg bw. Group 5 remained untreated as controls. Groups were slaughtered 3, 7, 10, and 21 days after treatment. Four animals (2 in the 21-day group, 2 in the control group) were treated during the study with Terramycin foot spray against interdigital dermatitis. Tissue samples (pooled subcutaneous, mesenteric and renal fat, gluteal muscle, liver and kidney) were stored at –20°C (maximum 39 days), and analysed by LC-MS-MS method CLE 808/185-04R for diflubenzuron. Residues were not corrected for concurrent method recoveries (67%-83%) or matrix interferences (<0.004 mg/kg for fat and liver, <0.005 mg/kg for muscle, max 0.0011 mg/kg for kidney), see Table 72.

The results are shown in Table 139. Fat contained the highest residues (max. 0.28 mg/kg 3 days after treatment), followed by muscle (max. 0.17 mg/kg). Residues were below the LOQ (0.05 mg/kg) in fat and muscle after 10 days and in liver and kidney at all times.

Table 139. Diflubenzuron residues in ovine tissues after pour-on treatment.

Waiting period (days)	Diflubenzuron, mg/kg				
	Sheep no	Fat	Muscle	Kidney	Liver
3	1M	0.26	0.070	<LOQ	<LOQ
	2M	0.28	0.17	<LOQ	<LOQ
	3F	0.23	0.15	<LOQ	<LOQ
	4F	0.075	0.084	<LOQ	<LOQ
7	5M	0.12	0.10	<LOQ	<LOQ
	6M	0.059	0.10	<LOQ	<LOQ
	7F	0.085	0.12	<LOQ	<DL
	8F	0.20	0.13	<LOQ	<DL
10	9M	<LOQ	<LOQ	<LOQ	<DL
	10M	<LOQ	<LOQ	<LOQ	<DL
	11F	<LOQ	<LOQ	<LOQ	<LOQ
	12F	<LOQ	<LOQ	<LOQ	<LOQ
21	13M*	<LOQ	0.067	<LOQ	<DL
	14M	<LOQ	<LOQ	<LOQ	<DL
	15F	<LOQ	<DL	<LOQ	<DL
	16F*	<LOQ	0.079	<LOQ	<DL
control 3 days	17M	<DL	<DL	<DL	<DL
control 21 days	18M*	<DL	<DL	<LOQ	<DL
control 3 days	19F*	<DL	<DL	<DL	<DL
control 21 days	20F	<DL	<DL	<LOQ	<DL

na: not analysed
ns: not specified
* treated additionally with terramycin foot spray during the study
DL (detection limit) 0.005 mg/kg in muscle, 0.004 mg/kg in liver and fat, 0.0003 mg/kg in kidney
LOQ 0.05 mg/kg for all tissues

Fish. The Meeting was informed by the applicant that there was no need to support a Codex MRL for salmon, because little of the product is sold in Norway and there is already an EU veterinary MRL. According to the applicant, the pre-slaughter interval (60 days) will prevent the presence of quantifiable residues in exported salmon.

Farm animal feeding studies

Animal feed-through applications are registered for pigs and poultry. Animal feeding studies were reported for cattle, sheep and chickens.

Cattle. An air-milled formulation of diflubenzuron was premixed with a feed concentrate and added as supplement to the feed of dairy cows (Miller *et al.*, 1976a, non-GLP). Control cows were not included. One cow was fed diflubenzuron at 1 mg/kg bw per day for 119 days, and another at rates increasing from 1 to 8 mg/kg bw/day for 2-week periods and after 56 days at 16 mg/kg bw/day for 94 days. The amount of diflubenzuron in the total daily ration was not reported and could not be calculated as total feed intakes and body weights were not reported. Milk samples were taken from the second cow after each increase in rate. Both cows were slaughtered on the final day of feeding. Samples of kidney, liver, muscle, renal fat, omental fat, diaphragm fat and subcutaneous fat were stored frozen (temperature and time not reported). Milk and tissues were analysed for diflubenzuron by HPLC method B. Residues were not corrected for a mean recovery of 93-94% (Table 62). Tissues from the second cow were reanalysed at another laboratory (Beltsville) by another method (Oehler *et al.*, 1974, non-GLP, full reference not available). The reported LOQ was 0.1 mg/kg at both laboratories.

No diflubenzuron was found in milk (reported LOQ 0.02 mg/kg) during the period when 1-8 mg/kg bw per day was fed, but 0.02 mg/kg was found when the rate was increased to 16 mg/kg bw/day. The maximum residue found was 0.25 mg/kg diflubenzuron in body fat (Table 140). Results at the Beltsville laboratory (BV) were higher than at the College Station laboratory (CS), and only the Beltsville results are shown in Table 140.

Table 140. Diflubenzuron residues in milk and tissues of cows after feeding with diflubenzuron.

Cow	Feeding level, mg/kg bw	Diflubenzuron, mg/kg								
		lab	milk	muscle	liver	kidney	renal fat	omental fat	diaphrag-matic fat	subcutaneous fat
1652F	1 for 119 days	CS	na	<0.1	<0.1	<0.1	<0.1	0.10	<0.1	<0.1
5036F	1-8 for 56 days; 16 for 94 days	CS	<0.02 0.02	<0.1	0.10	<0.1	0.10	0.10	0.10	0.10
5036F	16 for 94 days	BV	na	<0.1	0.13	<0.1	0.20	0.20	0.25	0.20

na: not analysed

Diflubenzuron was added at a nominal rate of 100 mg/kg to a supplement to the feed of eight Holstein bull calves (Miller *et al.*, 1979, non-GLP). The calves were divided into four pairs each consisting of a control and a treated animal. One calf was fed 2.8 mg/kg bw diflubenzuron per day from 3 days of age until slaughter at 146 days and three were fed 2.8 mg/kg bw per day from 3-208 days of age and thereafter with 1.0 mg/kg bw per day until slaughter at 349, 569 or 571 days. The amount of diflubenzuron in the total daily ration was not reported and could not be calculated as total feed intakes were not reported. The initial weights of the animals were not reported; the body weight gain was 983-995 g/day. Samples of liver, kidney, muscle and renal, omental and subcutaneous fat were stored at –20°C (time not reported) and analysed for diflubenzuron by HPLC method B. No peaks were detected in control samples and the mean recovery was 108% (Table 62).

Diflubenzuron was not found in any of the tissues analysed, except in calf 2M slaughtered at 146 days of age (Table 141).

Table 141. Diflubenzuron residues in tissues of calves after feeding with diflubenzuron.

Calf	Feeding level mg/kg bw	Diflubenzuron, mg/kg					
		muscle	liver	kidney	renal fat	omental fat	subcutaneous fat
2M	2.8 for 143 days	<0.02	0.02	0.02	0.08	0.04	0.04
4M	2.8 for 205 days 1.0 for 141 days	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
6M	2.8 for 205 days 1.0 for 361 days	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
8M	2.8 for 205 days 1.0 for 363 days	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02

In the USA Vigilante® 0.77% diflubenzuron feed-through premixed with a feed concentrate added at a nominal rate of 77 mg/kg to a supplement to the feed of cattle (Romano, 1985, non-GLP).

Three Hereford bulls and three Hereford cows received 0.2 mg/kg bw diflubenzuron with their daily ration over a period of 28 days, with two bulls and one cow as controls. The average weight was 319 kg (range 284-370 kg). The amount of diflubenzuron in the total daily ration was not reported and could not be calculated as total feed intakes were not reported. The animals were slaughtered between 3 and 8 hours after the feed. Samples of liver, muscle, kidney and fat from each animal were frozen (temperature and storage time not reported) and analysed for diflubenzuron by HPLC method M-1590. Control residues were <0.015 mg/kg: concurrent recoveries were not determined. One liver sample contained 0.06 mg/kg (Table 142) but all other samples were below the reported LOQ of 0.05 mg/kg. Metabolites were not determined.

Table 142. Diflubenzuron residues in tissues of cattle at a feed level of 0.2 mg/kg bw diflubenzuron for 28 days.

Cattle	Diflubenzuron, mg/kg			
	muscle	liver	kidney	fat
25M, 51M, 52M	<0.05, <0.05, <0.05	<0.05, <0.05, 0.06	<0.05, <0.05, <0.05	<0.05, <0.05, <0.05
70F, 73F, 87F	<0.05, <0.05, <0.05	<0.05, <0.05, <0.05	<0.05, <0.05, <0.05	<0.05, <0.05, <0.05

The same product was added in the same way at the same rate to determine residues in milk (Garces, 1985, non-GLP). Nine lactating Holstein cows, weighing on average 548 kg (range 471-635 kg), were divided into three control cows and six cows which received 0.2 mg/kg bw diflubenzuron with their daily ration for 28 days. The amount of diflubenzuron corresponded to 2.4-4.4 ppm diflubenzuron in the feed (calculated by the reviewer from actual feed intakes). Milk samples were collected from each cow 3, 7, 14, 21 and 28 days after treatment and analysed by HPLC method M-1593. Control samples contained <0.003 mg/kg diflubenzuron, concurrent recoveries were not determined. Storage conditions were not reported. The diflubenzuron residues in all the samples were below the reported LOQ of 0.01 mg/kg at each sampling. Metabolites were not determined.

Sheep. Columbia-Rambouillet sheep were fed diflubenzuron as the technical material incorporated into the feed at 100 ppm (Escobar, 1978, non-GLP). Feeding started one month before breeding and continued until 1-2 months past birth of the lambs. Ewes were slaughtered 1, 3, 4, 5, 6 and 9 months after feeding stopped, and males after 7 months. Milk samples were taken 0, 2, 4, 5, 6 and 8 weeks after lactation. Ewes were returned to a control ration when their lambs were weaned and slaughtered at 1, 2 or 4-week intervals to study depletion rates. Lambs were fed 12.5, 25, 100 or 250 ppm diflubenzuron in the diet for 4-10 weeks. Samples were stored at 0°C for 180-300 days, when some of the kidney samples were analysed for diflubenzuron by HPLC method AM-3 and all other samples by GC-ECD method AM-15A. The results (Tables 143-145) were corrected for concurrent method recoveries (72%-123%), but not for matrix interferences (<0.05 mg/kg in control samples) (Tables 63 and 80).

Table 143. Diflubenzuron residues in tissues of sheep fed with 100 ppm diflubenzuron in the feed.

Sheep no	Duration of feeding	Diflubenzuron, mg/kg			
		muscle	liver	kidney	fat
17	1 months	0.09	0.40	0.11[1]	0.96
47	3 months	0.08	0.51	0.12[1]	1.4
36	4 months	0.06	0.58	0.09[1]	1.1
30	5 months	<0.05	0.42	<0.05[1]	0.86
16	6 months	0.18	0.56	0.10[1]	0.96
49	7 months	0.07	0.15	0.09[1]	0.78
01, 04, 05	9 months	0.07, <0.05, <0.05	0.18, 0.21, 0.16	0.22, 0.15, 0.10	1.1, 0.41, 0.26
06, 18, 23	9 months	0.26, <0.05, <0.09	0.08, 0.13, 0.18	0.33, 0.07, 0.11	1.7, 0.35, 0.59
27, 40, 48	9 months	<0.05, <0.05, <0.05	0.10, 0.25, 0.23	0.05, 0.12, 0.17	0.36, 0.67, 1.0
15	- 1 week	<0.05	<0.05	<0.05	0.18
06???	- 2 weeks	<0.05	0.45	0.06	0.20
12	- 4 weeks	<0.05	<0.05	<0.05	0.12

[1] analysed by HPLC method AM-3

Table 144. Diflubenzuron residues in tissues of lambs fed for 4-10 weeks with the indicated diflubenzuron level in the diet.

Lamb no	Feeding level, ppm	Duration of feeding	Diflubenzuron, mg/kg			
			muscle	liver	kidney	fat
85	12.5	4 weeks	<0.05	<0.05	<0.05	0.09
73	12.5	10 weeks	<0.05	<0.05	<0.05	0.20
84	25	4 weeks	<0.05	0.06	0.05	0.21
66	25	10 weeks	<0.05	<0.05	<0.05	0.06
88	100	4 weeks	0.14	0.43	0.36	1.3
79	100	10 weeks	<0.05	0.19	0.09	0.19
74	250	4 weeks	<0.05	0.19	0.20	0.66
80	250	10 weeks	0.07	0.47	0.75	2.4

Table 145. Residues in milk of ewes fed with 100 ppm diflubenzuron in the diet.

Sheep no	Week of lactation	Diflubenzuron, mg/kg
12, 15, 35, 37, 51	2	0.23, 0.37, 0.34, 0.32, 0.44
12, 15, 35, 37, 51	4	0.27, 0.13, 0.24, 0.42, 0.27
12, 35, 37	6	0.23, 0.34, 0.28
12, 35, 37	8	0.37, 0.32, 0.34

Chickens. Diflubenzuron was fed to 8 White Leghorn and 8 Black Sexlinked Cross hens at a level of 10 ppm in the feed (0.56-0.61 mg/kg bw) for 15 weeks (Miller *et al.*, 1976b, non-GLP). Control hens (8 of each breed) received the ration without diflubenzuron. Eggs were collected daily for the first 21 days and thereafter once a week. After 11 weeks treatment all hens were artificially inseminated once a week for 4 weeks and eggs were collected daily. After 15.5 weeks treatment the hens were killed and samples taken of breast muscle, liver and visceral fat. Storage conditions were not reported. Eggs were analysed by a method not available to the reviewer (Miller *et al.*, 1975). Tissues were analysed for diflubenzuron by HPLC method B. Validation data were not available.

Residues in eggs, liver and visceral fat were significantly in White Leghorns than in Black Sexlinked Cross; no residues were found in muscle (Table 146). Individual residue values for chickens were not reported. Residues of diflubenzuron started to accumulate in eggs the 4th day of feeding.

Table 146. Mean residues of diflubenzuron (± SD) in eggs and tissues of chickens.

Breed	Diflubenzuron, mg/kg				
	intake (mg/kg bw/day)	Eggs 2nd-9th week	Muscle	Liver	Visceral fat
Black Sexlinked Cross	0.61±0.005	0.38	<0.1	0.12±0.021	1.2±0.11
White Leghorn	0.56±0.005	0.53	<0.1	0.45±0.021	1.8±0.11

Differences between treatment with 0, 2.5 mg/kg and 250 mg/kg of diflubenzuron in the feed were investigated in male Hubbard broilers in a randomised block trial with 16 blocks (De Wilde and Buisman, 1976, non-GLP). Each block contained 4 pens (2 controls and one of each dose of diflubenzuron) and each pen contained 24 birds. After 98 days 5 birds from each of 5 pens per dose group were killed for tissue analysis, and their fat, breast muscles + covering skin, leg muscle and livers were pooled and stored at -20°C (storage time not reported). Analysis for diflubenzuron was by HPLC method LAI 3-86-5 (description not available to the reviewer). Results were not corrected for recoveries (76%-116%) or matrix interferences (0.12 mg/kg in fat, 0.024 mg/kg in muscle, 0.06 mg/kg in liver). Because of high control residues, levels below 0.4 mg/kg diflubenzuron in fat, 0.08 mg/kg in muscle and 0.05 mg/kg in liver are not considered valid.

Diflubenzuron concentrations in the tissues are shown in Table 147. A 100-fold higher dose in the diet yields an increase in tissue residues of only a 7-fold. Diflubenzuron was mainly found in the fat.

Table 147. Residues of diflubenzuron in pooled poultry tissues after 98 days of feeding.

Pen no.	Feeding level	Diflubenzuron, mg/kg[1]			
		Breast muscle	Leg muscle	Liver	Fat
1, 13, 31, 48, 55	2.5	0.22, 0.25 0.27, 0.31, 0.098, mean 0.24	0.29, 0.19, 0.41, 0.38, 0.13, mean 0.30	0.45, 0.27 0.67, 0.70, 0.11, mean 0.43	5.8, 4.0, 6.3, 5.3, 2.0, mean 5.1
12, 22, 32, 46, 56	250	2.9, 2.5, 1.6, 1.2, 0.99 mean 2.1	2.8, 2.4, 1.2, 1.2, 0.85 mean 1.9	3.5, 3.4, 1.2, 1.0, 0.73 mean 2.1	56, 43, 26, 26, 21 mean 38.2

[1] Pooled tissues of 5 birds

In The Netherlands, Vigilante® 0.73% diflubenzuron feed-through was mixed with chicken feed at a nominal level of 10 ppm diflubenzuron in the feed (Buisman and Verhaar, 1985b, De Boer, 1993, both non-GLP). The actual feed content was 7.2-8.7 ppm (average 7.7). White Leghorn Shaver 288 and brown Warren laying hens (10 of each strain) were fed for 28 days with this mixture. Control hens (20 of each strain) received feed without diflubenzuron, and egg samples were taken. After 28 days the hens were killed and samples of subcutaneous fat, muscle (1:1 mix of chest and leg), kidney, and liver were taken. Sample storage conditions were not reported. HPLC method L3-86-17 was used for egg white and yolk and HPLC method E for the tissues. Results were not corrected for recovery (71%-89% in eggs, 59%-95% in tissues) or matrix interferences (<0.012 mg/kg) (Tables 65 and 69).

White hen tissues and eggs contained higher residue levels than brown. Both strains had the highest level of diflubenzuron in fat. Results are shown in Table 148.

Table 148. Diflubenzuron residues in tissues of white and brown hens after feeding 10 mg/kg diflubenzuron in the feed for 28 days.

Sample	white hens; mg/kg[1]			brown hens; mg/kg[1]		
	day 14	day 21	day 28	day 14	day 21	day 28
Egg yolk	1.9	1.6	1.4	0.95	0.99	0.83
Egg white	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
Whole egg[1]	0.65	0.58	0.48	0.33	0.35	0.29
Muscle[2]			0.14			0.065
Liver[2]			0.47			0.13
Kidney			0.17			0.060
Fat[2]			2.3			1.4

[1] calculated from yolk residue; 35% yolk in egg without shell; egg white did not contain diflubenzuron (<0.04 mg/kg)
[2] means of 3 analytical replicates from pooled tissues of 4 hens

RESIDUES IN FOOD IN COMMERCE OR AT CONSUMPTION

No information available.

NATIONAL MAXIMUM RESIDUE LIMITS

The Meeting was informed by the company of the following national MRLs. MRLs for Australia were provided by the government.

National MRLs for food crops and animal commodities. The residue is defined as diflubenzuron in all countries listed.

Country	Sample	MRL (mg/kg)	Notes
Australia	mushrooms cereal grains wheat bran, unprocessed pastures (mixed grasses, leguminous) cattle meat cattle, edible offal of cattle milk sheep kidney sheep liver sheep meat (in the fat) sheep milk	0.1 2T 5T 50T (fresh weight) 0.02* 0.02* 0.05 0.05 0.05 0.05 0.05	* at or about the LOQ T = temporary MRL
Belgium	pome fruit cabbage crops mushroom other food commodities	1 1 1 0.05*	* at or about the LOQ
Brazil	soya bean cotton	0.2 0.2	
France	pome fruit walnut	1 0.05	
Germany	apple, pear wild forest berries cabbage mushroom other edible crops tobacco (import tolerance)	1 2 1 0.02 0.05 100	
Hungary	apple, pear cherry, sour cherry nectarine, peach, plum mushroom cereals (grain, straw)	1 0.5 0.1 0.1 0.1	
Italy	pome fruit mushroom	1 0.2	
Netherlands	pome fruit flowering brassicas head brassicas leafy brassicas fungi other food commodities	1 1 1 1 0.1 0.05*	* at or about the LOQ
Russia	apple	0.1	
Spain	citrus fruit other fruits mushroom other food commodities	1 1 0.1 0.05	
Turkey	apple, pear peach	1 1	
UK	citrus fruit pome fruit plum Brussels sprouts, head cabbage tomato, pepper, aubergine cultivated mushroom meat milk egg	1 1 1 1 1 0.1 0.05 0.05 0.05	
Yugoslavia	apple, pear sugar beet	1 0.1	

APPRAISAL

Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] is included in the CCPR periodic review programme. This insecticide was originally evaluated by the JMPR in 1981 and re-evaluated for residues several times up to 1988. At the 28th Session of the CCPR in 1996 (ALINORM 97/24) diflubenzuron was scheduled for the JMPR in 1999 as a priority compound under the Periodic Review Program. However, the manufacturer asked for a postponement and therefore the periodic review of diflubenzuron was re-scheduled for the JMPR in 2002.

The primary manufacturer supplied information on identity, metabolism and environmental fate, residue analysis, use pattern, residues resulting from supervised trials on crops (almonds, apples, berries, blackcurrants, Brussels sprouts, Chilli peppers, cotton, gooseberries, grapefruits, head cabbages, lemons, limes, mandarins, mushrooms, oranges, peaches, pears, peas, pecans, plums, range grass, rice, soybeans, sweet peppers, tomatoes, walnuts), fate of residues during storage or in processing, residues in animal commodities (meat, milk, eggs) resulting from direct animal treatment or feeding, and national MRLs. In addition, GAP information and National MRLs were supplied by The Netherlands, Germany and Australia.

Animal metabolism

The Meeting received information on the fate of orally dosed diflubenzuron in lactating cows, male sheep, lactating goats, laying hens, and pigs and on dermally applied diflubenzuron on cattle. Studies on laboratory animal metabolism (rat, mouse, rabbit, cat) were evaluated by the WHO panel of the 2001 JMPR. All studies were performed using ^{14}C-diflubenzuron, equally labelled in both phenyl moieties.

The studies indicated that diflubenzuron is metabolised via two routes. Hydroxylation of the phenyl groups, which leaves the basic structure of diflubenzuron intact, yields the metabolites 2,6-difluoro-3-hydroxydiflubenzuron (3-OH(F)-DFB), 4-chloro-3-hydroxydiflubenzuron (3-OH-DFB), 4-chloro-2-hydroxydiflubenzuron (2-OH-DFB) and their conjugates. On the other hand, cleavage between the carbonyl and amide groups yields 2,6-difluorobenzoic acid (DFBA), 2,6-difluorobenzamide (DFBAM) and p-chlorophenylurea (CPU).

Lactating cows and goats excreted 73-86% of the ^{14}C administered in the faces and 4-15% in the urine. Into the milk 0.07-0.2% was secreted. In muscle and fat, no radioactive residues could be detected. In liver, 0.4-0.8% of the administered dose was found and in kidney 0.01-0.02%. Radioactivity in liver could be attributed to the following components: parent (3.5-7.0% TRR; both cow and goat), DFBA (13-20% in cow), DFBAM (1-5% in goat), CPU (0.2% in cow, 11-16% in goat), p-chloroaniline (PCA; 1.4% in cow, maybe also in goat at low amounts). The nature of the residue in kidney was not investigated.

In both cow studies, 61%-82% TRR could be extracted from the milk with acidified ethyl acetate. Concerning the nature of the residue in milk, conflicting data were reported. In the first cow study, it is stated that the radioactivity present in milk was not due to the parent itself, but to non-specified metabolites. However, in the second cow study, a significant amount of parent compound (43% TRR) was found in milk and metabolites were identified as well: DFBAM (13% TRR), 3-OH(F)-DFB (12% TRR) and 2,6-difluorohippuric acid (DFHA; 2% TRR). In the goat study, 87% TRR could be extracted from milk with 10% ammonia. HPLC analysis of milk extracts from goat showed, that the residue in milk consisted of about 8 components. None of these components was parent, CPU, PCA or p-chloroacetanilide (PCAA). About 20% of the metabolites was characterised as sulphate- or glucuronide conjugates.

^{14}C-Diflubenzuron was not degraded to any significant extent when incubated *in vitro* with digestive tract fluids of cattle or sheep.

Pigs rapidly excreted 70-80% of the radioactive oral dose in the faeces, and 5-10% in the urine. Six hours after the last dose, radioactive residues in muscle and fat were below the LOQ, in liver and kidney low levels of radioactivity were detectable. Diflubenzuron itself could not be detected in liver and kidney. The main metabolites in liver and kidney were found to be DFBA (30 and 55%, respectively) and DFHA (20 and 10%, respectively).

Laying hens showed rapid elimination of radioactivity in excreta: 40-65% of the administered dose in the first 8 hours after administration. In total, 80-90% of the administered dose was recovered in the excreta. About 4% was recovered from the tissues. Of the relevant tissues, the highest residue levels were present in fat and (partially formed) eggs, followed by liver and kidney while only minor amounts were found in muscle. In chicken eggs, 0.30-0.79% of the dose is excreted.

In chicken fat, 99% of the radioactive residue could be attributed to parent compound. In muscle, 63-76% was parent, about 13-22% CPU, and about 8% DFBA. In liver, 19-49% was parent, 20-50% CPU, about 7% DFBA, 1-3% PCA and about 3% PCAA. In kidney, 12-24% was parent, 23-40% CPU, and about 4% was PCA. In eggs, 69-80% of the radioactive residue was found to be parent, 11% CPU, and 4% DFBA. Traces of PCAA were found in one dose group. Almost all residue was present in egg yolk, negligible amounts were in the egg white.

Studies with a stanchioned, catheterised cow indicated that diflubenzuron applied as WP is not absorbed through the skin to any significant extent after dermal application. During a 3 day period after application, no detectable residues were excreted in the urine. After 3 days, 68% of the radioactivity applied was recovered by clipping and extracting the treated hair and thoroughly washing the exposed skin with acetone. TLC of these fractions showed that DFB was the only radioactive compound found. Residues in tissues were not investigated.

Metabolism of diflubenzuron in laboratory animals was qualitatively comparable to that in farm animals.

Plant metabolism

The Meeting received information on the fate of diflubenzuron after spotwise treatment of leaves from maize, soybean, cabbage, apple, cotton and rice, after application to fruits of apple and orange, after application to pods of soybean, after soil application to cotton, after surface water treatment to rice and wheat, after compost and/or casing treatment to mushrooms. Further, information was received on the fate of diflubenzuron after incubation on bean leaf disks and after injections into the stem and leaves of lima bean, and on CPU and DFBA uptake by root/stems from nutrient solutions. The studies were conducted with diflubenzuron labelled in both rings with ^{14}C, or labelled with ^{14}C (chloroaniline ring) and ^{3}H (difluorobenzoyl ring) in the same molecule or with a mixture of ^{14}C-diflubenzuron labelled at the chloroaniline and the difluorobenzoyl moiety.

After spotwise treatment of leaves from apple, maize, soybean, cabbage, >90% of the recovered radioactivity was found to be parent compound. The residue did not translocate. Spotwise treatment of apples and oranges (the fruit) gave the same result. Spot wise treatment of developing pods of soybean plants showed >99% of the recovered radioactivity in the treated pods and less than 0.2% in the untreated parts (vines, untreated pods). On the treated pods, >99% of the recovered radioactivity was found in the hulls and less than 0.2% was found in the seeds. In the immature pods and mature hulls 90%-104% TRR was identified as parent compound.

Miniature citrus trees (in a greenhouse) were sprayed with radiolabelled diflubenzuron. Two simulated rain events removed 57%-87% TRR. When treated citrus leaves were soaked in tap water for 24 hours, essentially quantitative removal of radioactivity was observed (96% TRR).

In soybean plants treated twice at mid to full bloom (foliar treatment), diflubenzuron residues were found in foliage from 0-8 weeks. At maturity (after 12 weeks) residues were found in trash, leaves, pods, hulls, but not in seeds. It was found that 57%-100% TRR was extractable and this was observed to be unchanged diflubenzuron.

Agar cylinders containing ^{14}C-diflubenzuron were placed on dwarf bean leaf disks for 24 hours, of which 16 hours under illumination and 8 hours in the dark. No blackening of the X-ray film was observed outside the spots where the agar cylinders had been, nor on the places where the epidermis + cuticula were removed. This indicates, that ^{14}C-diflubenzuron does not penetrate the leaf disks.

LSC analysis of bean plants following stem injection of diflubenzuron, revealed that 84% of the applied radioactivity remained in the stems, the leaves contained 9% and the roots 0.2%. In the stems 89% - 88% TRR consisted of parent, up to 12 days after application. In the leaves (6 days after exposure), the organosoluble fraction consisted of parent (1.3% TRR), 2-OH-DFB (2.4%), 3-OH-DFB (8.9%), DFBAM (0.8%), CPU (0.7%), DFBA (0.6%) and 2 unknowns (1.7% and 0.2%). PCA was not detected.

In a greenhouse, leaves of cotton plants were sprayed with radiolabelled diflubenzuron. Of the applied radioactivity 100% was recovered in the treated leaves, 0.18%-0.37% in the bolls and squares, <0.01%-0.08% in stems, roots and new growth. When bolls and squares were subdivided in burr, seed and cotton fibre, the radioactivity was mainly present in the burr. In cotton seed extracts no radioactivity was found. The ^{14}C in the leaf and stem extracts was identified as diflubenzuron.

Cotton plants (41 days old) were transplanted in diflubenzuron-treated soil and 89 days after soil treatment 51% of the applied radioactivity was present in the soil; 3.5% in the plants and 46% was missing (probably degraded to $^{14}CO_2$). After 89 days radioactive residue was found in leaves (67%), roots (24%), stems (4.5%), and bolls plus squares (2.8%).

The ^{14}C in the 89 day soil extract was characterised as parent (13% TRR), CPU (10% TRR), DFBA (3%-4% TRR). The extractable ^{14}C from leaf samples was identified as CPU (21% TRR). The extractable ^{14}C from root samples was characterised as parent (major part) and DFBA (minor part).

In the field, separate leaves of cotton plants were treated with radiolabelled diflubenzuron. After 14 days, 87% of the applied radioactivity could be washed off by organic solvents. After 21 days and following a rainfall, 70% of the applied diflubenzuron was washed off. After 28 days exposure to summer sunlight (protection against rainfall; crop oil suspension), 38% was lost as a result of volatilization. In extracts from cotton leaves, only the parent compound was found, no degradation products were observed.

Rice and wheat plants (in a greenhouse) were treated with radiolabelled diflubenzuron (^{14}C and ^{3}H) added to the irrigation water. Of the total recovered radioactivity 88%-94% (both ^{3}H, ^{14}C) was found in the soil, 1.3% (^{14}C) and 10% (^{3}H) was found in the roots and 6.2%-10% (^{14}C) and 1.8%-6.3% (^{3}H) was found in the shoots. The ^{3}H residues were not characterized (DFB-DFBA route). In the soil parent was present at 25% TRR up to 2 weeks after application and at 1.4%-9.9% up to 18 weeks after application. CPU was found in the soil at 0%-53% TRR. In the rice leaves 0%-16% TRR was identified as parent compound; CPU was found at 0%-72%. In the wheat leaves, parent compound was not detected; CPU was found at 39% TRR). In wheat grain, neither parent nor CPU was found.

Rice plants (in a greenhouse) received a foliar spray of radiolabelled diflubenzuron (1:1 mixture of ^{14}C ring labels). Only a very small amount of the applied radiolabel moved from the foliage to the grain. In rice grain 26%-32% TRR was extractable; CPU was identified as the major metabolite (17%-22% TRR); minor residues were parent (0.2%-0.3%), CPU conjugates (0.9%), DFBA conjugates (3.0%), PCA (0.3%) and unknown compounds (5.0%-9.4%). The non-extractable residues in rice grain were characterized as ^{14}C incorporated into glucose units of starch (30% TRR), into protein (12%) or as bound or lignin related residues (24%). Hydrolytic treatments released 5.0%-35% TRR from the non-extractable residues: no residues of diflubenzuron or its primary metabolites could be detected in the hydrolysates.

In rice straw 71%-81% TRR was extractable; parent (36%-42% TRR) and CPU (26%-29%) were the major residues; minor metabolites were CPU conjugates (2.5%), DFBA conjugates (2.1%), PCA (0.2%) and unknown compounds (5.8%-8.6%). The non-extractable residues in rice straw could be released by acid/base hydrolysis (15% TRR), resulting in CPU as the major metabolite (10%) and DFBA (2.2%), PCA (0.4%) and unknown compounds (2.2%) as minor metabolites.

The compost and casing layer of mushrooms were subsequently treated (indoors) with radiolabelled diflubenzuron. The main metabolites in the growth medium were CPU (25%-38% TRR) and DFBA (10%-33% TRR). PCA was present in amounts <1% TRR. Diflubenzuron applied to the casing is metabolized more rapidly than diflubenzuron applied to the compost.

The amount of parent compound was highest in the first flush of mushrooms (8.2%-17% TRR; 19 days after last treatment) and decreased to levels at or below the LOQ at subsequent flushes. The main metabolites in mushrooms in one study were CPU (54%-82% TRR) and DFBA (25%-43% TRR). PCA was present in amounts <1% TRR at day 32. Distillation of mushroom extracts indicated that 40%-70% TRR was possibly tritiated water. In another study, the main part of the residue in the mushrooms (compost and casing treatment) consisted of DFBA (81%-88% TRR).

CPU uptake from nutrient solutions was tested on tomato and broad bean plants. CPU was rapidly taken up by the roots and transported via the xylem to the leaves. CPU accumulated in the leaves and was metabolized to PCA at very slow rates.

DFBA uptake from nutrient solutions was tested on tomato plants. DFBA was decarboxylated rapidly under the influence of tomato roots and the xylem sap contained very little DFBA.

The Meeting concluded that the metabolism and degradation of diflubenzuron on crops is adequately understood. The compound does not penetrate into plant tissue and residues are only present on those parts directly exposed during the application. After application to aerial parts of plants, diflubenzuron is not metabolized to any practical extent. Diflubenzuron can be partly washed off by rainfall or can be volatilised by sunshine.

When applied to bare soil, diflubenzuron is partly degraded to CO_2. When plants are growing in the same soil, a larger part of diflubenzuron is degraded to CO_2 and low amounts of residues are found in the plant. Parent and the soil metabolites CPU and DFBA can be taken up by the roots: parent and DFBA remain in the roots, CPU is translocated to the leaves. Therefore, in rice and mushroom, CPU and DFBA are part of the residue.

All metabolites found in plants were also characterized in animal metabolism studies.

Environmental fate

Soil

Soil biodegradation. At the end of a laboratory study performed in a sandy loam soil at 24 °C for 21 days, unextracted radiolabel increased to 37% of the applied amount of ^{14}C-diflubenzuron while CO_2

formation increased to 26%. Four metabolites were identified. Except for CPU, all of them were found in amounts of <10% of the applied diflubenzuron. The amount of CPU increased to a maximum of 31% of the applied diflubenzuron after 7 days and decreased thereafter to 25% at day 21. From this study the half-live of diflubenzuron was calculated to be 50 hours at 24 °C, while the half-life of CPU in soil was calculated to be 43 days at 24 °C.

In a laboratory study in a loam and a sand soil, the biodegradation of DFBA was investigated. Soil bound residues at the end of the study after 32 days amounted to 37% and 33% of applied radioactivity in loam and sand, respectively, while CO_2 production increased to 28% and 52%, respectively. In loam, DFBA content in the extracts decreased from 98% on day 0 to 27% on day 32. From this a half-live of about 12 days was calculated. In sand, DFBA decreased from 96% on day 0 to 2% on day 32 from which a half-live of about 9 days was calculated.

Field dissipation half lives of 78 (application on citrus trees) and 11 (application of bare soil) days were obtained for diflubenzuron. Since DFBA and CPU were formed in very small amounts it was not possible to determine half-lives for these metabolites. Metabolites DFBA and CPU were found only in the upper 15 cm soil layer, with a maximum concentration of 0.04 mg/kg dry weight soil.

Two studies concerned with the photodegradation of diflubenzuron on soil layers were submitted. From one of the study, a half-live for photolysis of 68 days could be estimated.

Mobility of diflubenzuron in soils is very low. In laboratory batch adsorption experiments with eight different soils and sediments with organic matter (OM) contents of 0.56% to 4.8%, K_{om}s between 1920 and 12727 L/kg were obtained. There was no relationship between adsorption and clay content. Metabolite DFBA is very mobile: the sorption of this metabolite in three different soils was too low to calculate a reliable adsorption coefficient. Metabolite CPU is slightly mobile: K_{om}s between 123 and 171 L/kg were obtained in three different soil types (0.7% to 4.3% OM). K_{om}s for CPU obtained in a column leaching experiment were higher: values of ≥1548 and 276 L/kg were determined in sand and loam with 4.6% and 3.6% OM, respectively.

In a confined rotational crop study sandy loam soil was treated with a suspension of radiolabelled diflubenzuron (^{14}C and ^{3}H label). After an ageing period of 10 weeks, soybean and maize seedlings and potato tubers were planted in the soil. At harvest, 22-26 weeks after treatment, there were no extractable residues in the leaves, soybean seeds, maize cobs and potato tubers A low level of unextracted radiolabel was found in soybean leaves and seeds . In the maize leaves and cobs and in the potato leaves and tubers the total unextracted radiolabel was <0.005 mg/kg. In the plant extracts, traces of CPU, possibly parent and 2 unidentified metabolites were found.

In a field rotational crop study the bare soil was sprayed with radiolabelled diflubenzuron (^{14}C in both rings). Wheat, onion and cabbage were planted 2 months after the last treatment and were collected 5.5 months after the last treatment. Radioactivity in plant tissue was below the level of 0.01 mg/kg diflubenzuron eq.

In another field rotational crop study radiolabelled diflubenzuron (^{14}C in both rings) was sprayed onto field grown cotton. After harvest of the cotton, 90% of the cotton plant material was distributed over the surface area of the treated plots, and cultivated into the top 10 cm of soil. Wheat seed and collard seedlings were planted after 3 weeks, radish and pinto bean seeds were planted after 6 months. At harvest, radioactive residues were generally low in the rotational crops, especially in the edible portions.

Post-harvest residues of diflubenzuron in soil were located in the top 10 cm of the soil and were persistent during the subsequent winter and spring months, but declined slightly with the onset of high summer temperatures. In soil collected in spring the extractable residue was characterized as diflubenzuron (81% TRR), CPU (1.7%) and two unknowns (each <2%). In soil collected in the following autumn all extractable residue was identified as DFB.

The Meeting concluded that rotational crops take up very low amounts of residues. The Meeting observed that the persistence of diflubenzuron in soil in field studies is longer than as deduced from laboratory experiments (laboratory half life 2-3 days at 20 °C).

Water-sediment systems

In a 63 day study at 22 °C in the dark at pH 5, 7, 9, and 12 in sterile solutions, double labelled diflubenzuron hydrolyzed faster at higher pH. At pH 5, about 80% was remaining after 63 days, at pH 7 about 70%, and at pH 9 about 35%. At pH 12, 8% was remaining after 28 days. DFBA and CPU were identified as degradation products.

The photodegradation of ^{14}C-phenyl-labelled diflubenzuron was determined in a solution containing 1% acetonitrile irradiated for 15 days. After 15 days 85 % of the radioactivity was recovered, 78% of which was diflubenzuron. Metabolites DFBA, DFBAM and CPU were found in amounts of 4, 1 and 8% of the recovered radioactivity. PCA was not found.

Diflubenzuron is rapidly degraded in aerobic water/sediment systems. In a 45 day study with a sandy loam and a silty loam system, the half-life of diflubenzuron in the water phase was 2 and 1 days at 20 °C, respectively. The half-lives for the whole system were 25 and 10 days for sandy loam and silty loam, respectively. Metabolites DFBA and CPU were the major degradation products. In another study with a river and pond sediment, half-lives for diflubenzuron in the system were 5.4 and 3.7 days at 20 °C, respectively. Metabolites DFBA and CPU were formed in maximum amounts of 17% and 48% in the system. Indicative half-lives of CPU for the water phase were 18 and 32 days for river and pond, respectively, half-lives for the system are 27 and 53 days. The amount of sediment bound residues in the respective systems was 44% and 37% after 104 days, mineralization as CO_2 was 33% and 38% after 104 days in river and pond, respectively.

In an anaerobic system, the half-life of diflubenzuron in the water phase was 18 days at 20 °C, the half-live for the whole system is 34 days. DFBA and CPU were formed in the water phase in amounts of 39% and 26%, respectively, after 90 days.

In studies in ditch water, half-lives of 9 and 22 days were found at 25 and 24 °C. Metabolites DFBA and CPU were formed in maximum amounts of 34% and 42%.

Analytical methods

The Meeting received numerous analytical methods used in supervised residue trials or in studies on storage stability, environmental fate, processing, animal feeding or direct animal treatment. Most analytical methods are single methods for determinations of either diflubenzuron, DFBA, CPU, PCA or PCAA in only a few matrices. The sample clean-up is in most cases very laborious and has to be adapted for each matrix. In addition, the methods need modifications when the samples are aged due to the increase in matrix interferences thereby resulting in decreasing recoveries.

HPLC methods for diflubenzuron, CPU or PCAA consist of extraction, clean-up and direct determination by HPLC-UV, HPLC-MS or LC-MS-MS.

GC methods for diflubenzuron, CPU or PCA consist of hydrolysis of PCA conjugates, extraction, clean-up, hydrolysis of diflubenzuron, followed by derivatization with heptafluorobutyric acid anhydride and determination by GC-ECD or GC-MS. At the hydrolysis of diflubenzuron both CPU and PCA are formed, but both compounds are derivatised to the same product. Any CPU and

PCA present in the sample, will be determined as diflubenzuron if not separated prior to the hydrolysis step. PCA methods: when hydrolysis conditions for PCA are strong enough, any diflubenzuron or CPU present in the sample will be determined as PCA, if not separated prior to hydrolysis.

GC methods for DFBA consist of extraction (hydrolysis conditions), followed by clean-up and derivatisation with pentafluorobenzylbromide (PFBBr) or with diazomethane and determination by GC-ECD or GC-MS. When hydrolysis conditions for DFBA are strong enough, any diflubenzuron present in the sample will be determined as DFBA, if not separated prior to hydrolysis.

Since no hydrolysis is included, the proposed analytical methods in plants underestimate the amount of CPU present in the sample, as only the free CPU is determined and not the soluble/bound conjugates.

Enforcement methods (GC methods) were submitted for the single and separate determination of diflubenzuron (LAI 3-86-6), CPU (LAI 3-86-9) or PCA (PTRL 625W) in rice grain.

Reported LOQs for plant commodities generally range from 0.01-0.05 mg/kg, with exceptions going up to 0.1 mg/kg. However, because of high residue levels in control samples/ matrix interferences actual LOQs can be as high as 0.6 mg/kg. Reported LOQs for animal commodities range from 0.04-0.1 mg/kg. Recoveries in both plant and animal analytical methods were not always adequate and therefore results from trials with poor recoveries were excluded from evaluation.

Stability of pesticide residues in stored analytical samples

The Meeting received data on the stability of residues in plant products (grapefruits, lemons, limes, oranges, apples, pears, tomatoes, peppers, mushrooms, lettuce, turnip roots, wheat grain, wheat hay, rice commodities) and animal products (chicken manure, chicken muscle, chicken liver, chicken egg white, chicken egg yolk, cow’s milk, goat liver, goat milk) stored frozen.

Storage results for citrus fruits are conflicting. In the first study diflubenzuron residues in oranges and grapefruits decreased to 37%-71% when stored for 19 weeks at –20 °C. However, in 3 additional studies diflubenzuron was found to be stable in lemons, oranges, and limes when stored for 4 - 6 months at –10 °C.

Diflubenzuron residues in apples were stable for the time tested (1.5 months at –20 °C, and 7 weeks at –10 °C). In pears stored frozen for up to 12 months, diflubenzuron and CPU were stable for 3 months and PCA declined. Diflubenzuron storage was not investigated for longer storage times, CPU levels decreased to 32% after 6 months storage and PCA levels decreased to 47% after 1 month of storage.

Diflubenzuron residues in tomatoes were stable for the time tested (10 months) at –20 °C. In peppers stored frozen for up to 12 months, dilubenzuron and CPU were stable for 12 months and PCA was not stable. CPU levels decreased to a plateau level of 72%-75% after 3-12 months storage and PCA levels decreased to 59% after 1 month of storage.

In mushrooms stored frozen for up to 19 months, diflubenzuron was stable for 12 months, CPU was stable for 19 months, and PCA was not stable. Diflubenzuron levels decreased to 68% after 18 months of storage, PCA levels decreased to 14% after 1 month of storage.

In lettuce, turnip roots, wheat (grain, hay), rice (grain, bran, straw, hulls) stored frozen for up to 12 months, storage stability data for diflubenzuron and CPU were considered not validated beyond a 1 month time period, because of analytical problems. In this 1 month, diflubenzuron and CPU were stable. PCA levels were reduced within one month to 43% (lettuce), 78% (turnip roots), 68% (wheat grain), 69% (wheat hay), 40% (rice grain), 52% (rice bran), 67% (rice straw) or 65% (rice hulls).

In egg whites and cow's milk diflubenzuron was stable for 1 year at -20 °C. In another study goat milk and goat liver were fortified with a mixture of diflubenzuron, CPU, PCA and PCAA and were stored for 22 months at –10 °C. The analytical results showed a high variability (RSD>20%) and storage stability results from this study are considered not accurate.

Chicken liver, chicken thigh muscle, and egg yolk fortified with a mixture of diflubenzuron, CPU, PCAA and PCA, were stored frozen at –20, -80 and –195 °C. At all temperatures, diflubenzuron was stable for 10-15 months in egg yolk, chicken liver and chicken muscle. For CPU the best results were obtained at –80 °C or lower: in egg yolk, chicken liver and chicken muscle CPU was stable for 12 months. The storage results for PCA and PCAA are variable and both metabolites are considered not stable: low PCA levels tend to go together with high PCAA levels, perhaps from transformation of PCA in PCAA.

Residue definition

In farm animals, diflubenzuron was rapidly excreted. In ruminant and pig muscle and fat, radioactive residues were very low and could not be characterized. In chicken muscle, about 70% of the residue was parent, in chicken fat 99%. Liver of all farm animals except pig contained parent compound as one of the main residues. Kidney of ruminants was not investigated, chicken kidney contained parent as one of the main residues. The nature of the residue in milk is unclear; in one study 43% of the TRR was found to be parent, in two other studies parent was not detected. No major metabolite was identified in milk. In chicken eggs, a large part of the residue was parent, and almost all residue was present in the egg yolk.

The Meeting agreed that parent is a suitable marker molecule for enforcement in animal commodities and is also the compound of interest for dietary risk assessment.

The log K_{ow} of diflubenzuron is 3.89. Taking into account results from trials on direct animal treatment and farm animal feeding studies, the Meeting decided that diflubenzuron should be classified as fat-soluble.

In plants, diflubenzuron is a surface residue when applied to the aerial parts of the plant. The compound does not degrade nor translocate and can easily be washed of. Therefore in general diflubenzuron *per se* is the residue of interest both for enforcement and for dietary risk assessment.

However, in soil and water diflubenzuron is degraded to DFBA and CPU, which can be taken up by the plants. Thus in crops which grow on the treated soil (mushroom) or in flooded area (rice) these metabolites are present in larger quantities than the parent. Metabolism studies showed that the residue in rice grain consists mainly of CPU, and in rice straw of both CPU and diflubenzuron. In mushrooms DFBA and CPU are the main metabolites, and parent is mainly detected in the first flush.

Metabolism studies in rice and the USA supervised residue field trials available to this Meeting show that at the currently registered USA maximum dose rates, CPU levels are below the reported LOQ of 0.001 mg/kg in rice grain. In mushrooms, DFBA is the main residue, although the amount varies widely among studies. In view of the fact that DFBA is not a residue of particular toxicological concern and that the intake of mushrooms is quite low all around the world, and further that analytical methods for diflubenzuron, DFBA and CPU are quite laborious, the Meeting decided that the definition of the residue (for compliance with MRLs and for dietary intake) is diflubenzuron, both for plant and animal commodities. The residue is fat-soluble.

Results of supervised residue trials

Trials were available for citrus fruits (grapefruit, lemon, lime, mandarin, orange), pome fruits (apple, pear), stone fruits (peach, plum), berries (blackcurrants, gooseberries), brassica vegetables (Brussels sprouts, head cabbages), fruiting vegetables (sweet peppers, chilli peppers, tomatoes, mushrooms), pulses (peas, soybeans), rice, tree nuts (walnuts, almonds, pecans), cotton, and range grass.

Citrus fruits. Residue trials on citrus fruits were conducted in the USA (1985, 1988/1989, 1996), Spain (1995) and Italy (1996, 1997; no GAP). USA trials on grapefruits and oranges from 1988/89 could not be evaluated because of low storage stability results (37%-71% for diflubenzuron at 0.2-1.0 mg/kg). Italian trials on orange and lemon from 1997 could not be evaluated because of concurrent method recoveries as low as 46%.

Orange. Four USA trials (1985) with oranges were available. USA critical GAP is 3 times 0.35 kg ai/ha (interval 90 days) with a maximum spray concentration of 0.75 kg ai/hl (spray by aeroplane) or 0.075 kg ai/hl (spray). PHI is 21 days. One trial from 1985 was according to critical GAP, yielding a residue of 0.18 mg/kg.

Two Spanish trials (1995) with oranges were available. Spanish critical GAP is 0.015 kg ai/hL with a PHI of 30 days. Both trials complied with this GAP, yielding residues of 0.27 and 0.28 mg/kg.

Three Italian trials (1996) were available. Italy has no GAP for citrus, so the trials were evaluated according to the Spanish GAP. All 1996 trials were at GAP, yielding residues of 0.18, 0.27, 0.45 mg/kg.

Mandarin. Two Spanish trials (1995) with mandarins were at GAP, yielding residues of 0.18, 0.33 mg/kg.

Lemon. Two USA trials (1996) with lemon were available. The USA has no GAP on lemons. Three Italian trials (1996) were available. Italy has no GAP for citrus, so the trials were evaluated according to the Spanish GAP. All 1996 trials were at GAP, yielding residues of 0.18, 0.24, 0.26 mg/kg.

Lime. Two USA trials (1996) with lime were available. The USA has no GAP on lime.

Because residue results for single and double applications and residue results for different citrus fruits from USA, Italy and Spain are similar, residues were combined (STMR underlined): 0.18 (4), 0.24, 0.26, 0.27 (2), 0.28, 0.33, 0.45 mg/kg (lemon, mandarin, orange). Data on the residue in the edible portion were not available.

The Meeting agreed to maintain the current recommendation of 1 mg/kg for citrus fruit and estimated an STMR of 0.26 mg/kg for citrus whole fruit.

Pome fruit. Residue trials on apples and pears were conducted in The Netherlands (1974, 1975, 1976, 1979), Germany (1975, 1976, 1978, 1979, 1993), UK (1975, 1977, 1978, 1982), Poland, (1994), France (1974, 1975, 1976, 1979), Italy (1974, 1975, 1976, 1982, 1985, 1987), Spain (1975, 1976; no GAP), Japan (1976; no GAP), South Africa (1976/1977, 1977; no GAP), Canada (1983, 1984, 1997; no GAP) and the USA (1983, 1984, 1986, 1996, 1997; no GAP). South Africa, Canada and the USA have no registered use for diflubenzuron and the trials could not be evaluated against another GAP. Results from the 1976 German trials, the 1974 and 1976 Italian trials and the 1976 Spanish trials could not be used because of high values in control samples. Because residue results from 1974-1985 trials below 0.6 mg/kg are considered as not valid (matrix interferences), these results are expressed as <0.6 mg/kg.

Apples. Diflubenzuron is registered in The Netherlands for use on apples and pears as SC480 and WP250 formulation at 1-2 applications with a spray concentration of 0.01-0.02 kg ai/hL and a PHI of 14 days. Of the trials conducted in The Netherlands, Germany, UK, Poland and Northern France, 8 trials on apples (Netherlands 1974, 1976, Germany 1975) were conducted at the Dutch critical GAP, yielding residues of 0.14, 0.17, 0.27,- 0.31, 0.38, 0.40, 0.67, 0.89 mg/kg. Adjusted for matrix interference the residues are: <0.6 (6), 0.67, 0.89 mg/kg. .

Diflubenzuron is registered in Germany for use on apples and pears with WG 800 formulations at 1-4 applications at 0.18-0.30 kg ai/ha with normal spray at 0.012-0.02 kg ai/hL or low volume spray at 0.06-0.10 kg ai/hL with 14-21 day intervals and a PHI of 28 days. Of the trials conducted in Germany, The Netherlands, UK, Poland and Northern France, 1 trial on apples (Germany 1979) was conducted at the critical German GAP yielding a residue of 0.73 mg/kg.

Diflubenzuron is registered in the UK for use on apples and pears. Of the trials conducted in the UK, Germany, The Netherlands, Poland and Northern France, no trials were conducted at the UK critical GAP.

Diflubenzuron is registered in Poland for use on apples and pears with WP 250 formulations at 0.10-0.30 kg ai/ha with a PHI of 14 days at 0.0075-0.06 kg ai/hL. Of the trials conducted in Poland, UK Germany, The Netherlands and Northern France, 1 trial was conducted at the Polish critical GAP:. yielding a residue of 1.0 mg/kg for the application on apples (Germany 1979).

Diflubenzuron is registered in France for use on apples, pears, nashi pears and quinces with SC 150 and WP 250 formulations with a PHI of 15 days at 0.01 kg ai/hL. Of the trials conducted in France, Poland, UK, Germany, The Netherlands, Italy and Spain, residues complying with French critical GAP are: 0.043, 0.15, 0.19, 0.21, 0.22 (2), 0.23, 0.34 (2), 0.37, 0.38, 0.42, 0.66, 0.80 mg/kg for apples (France 1979, The Netherlands 1974, 1979, Poland 1994, Italy 1982, 1985, 1987). Residues adjusted for matrix interferences (1974-1985 trials) are: 0.043, 0.21, 0.34, <0.6 (9), 0.66, 0.80 mg/kg on apples.

Diflubenzuron is registered in Italy for use on apples and pears with WP 050 and WP 250 formulations with a PHI of 45 days at 0.01-0.02 kg ai/hL or 2 applications with a combination formulation with a PHI of 45 days at 0.006-0.012 kg ai/hl at an interval of 21 days. Of the trials conducted in Italy, Southern France and Spain, residues complying with Italian critical GAP are: 0.12, 0.28, 0.31, 0.42, 0.43, 0.47, 0.49, 0.57, 0.76, 0.92, 3.6 mg/kg on apples (Italy 1975, Southern France 1975, 1976, 1979, Spain 1975). Residues adjusted for matrix interferences are: <0.6 (8), 0.76, 0.92, 3.6 mg/kg for the applications on apples.

Diflubenzuron is registered in Spain for use on fruits with WP 250 formulations with a PHI of 30 days at 0.01-0.015 kg ai/hL. Of the apple trials conducted in Spain, Italy and Southern France, residues complying with Spanish critical GAP are: 0.15^F, 0.21^F, 0.28^I, 0.34^F, 0.43^I, 0.49^I, 0.60, 0.65^F, 0.92^I, 3.6^I mg/kg (Italy 1985, 1987, Southern France 1976, 1979). Results indicated with F or I were derived from trials where the same or a higher value was already selected for French or Italian GAP. Because only one residue per trial may be selected, the residues derived from the same trial (superscript F or I) were not considered for MRL estimation. Adjusted results are: 0.60 mg/kg for apples.

Diflubenzuron is not registered in Japan for use on pome fruit, but the residue trials can be evaluated against the GAP for China. Diflubenzuron is registered in China for use on apples with WP 250 formulations at 0.012-0.025 kg ai/hL. Of the trials conducted in Japan, residues complying with Chinese critical GAP are: 0.042, 0.11, 0.23, 0.40 mg/kg for applications on apples (Japan 1976). Because results below 0.05 mg/kg are considered not valid (matrix interferences), these results are expressed as <0.05 mg/kg. Corrected results are: <0.05, 0.11, 0.23, 0.40 mg/kg for applications on apples.

Pears. Diflubenzuron is registered in The Netherlands for use on apples and pears as SC480 and WP250 formulation at 1-2 applications with a spray concentration of 0.01-0.02 kg ai/hL and a PHI of 14 days. Of the pear trials conducted in The Netherlands, Germany, UK, Poland and Northern France, 2 trials were conducted at the Dutch critical GAP, yielding residues of 0.083, 0.11 mg/kg (UK 1982). Adjusted for matrix interferences the residues are: <0.6 (2) mg/kg.

Diflubenzuron is registered in France for use on apples, pears, nashi pears and quinces with SC 150 and WP 250 formulations with a PHI of 15 days at 0.01 kg ai/hL. Of the pear trials conducted in France, Poland, UK, Germany, The Netherlands, Italy and Spain, residues complying with French critical GAP are: 0.10, 0.12, 0.14, 0.33 mg/kg for pears (France 1979, Italy 1982, 1985). Adjusted for matrix interferences the residues are: <0.6 (4) mg/kg.

Diflubenzuron is registered in Spain for use on fruits with WP 250 formulations with a PHI of 30 days at 0.01-0.015 kg ai/hL. Of the pear trials conducted in Spain, Italy and Southern France, residues complying with Spanish critical GAP are: 0.29^F, 0.40 mg/kg (Italy 1985, Southern France 1979). The result indicated with F was derived from a trial where the same or a higher value was already selected for French GAP. Because only one residue per trial may be selected, this residue was not considered for MRL estimation. Adjusted for matrix interferences the remaining residue is: <0.6 mg/kg.

In conclusion, 40 trials on apples were selected yielding residues of 0.043, <0.05, 0.11, 0.21, 0.23, 0.34, 0.40, <u><0.6</u> (23), 0.60, 0.66, 0.67, 0.73, 0.76, 0.80, 0.89, 0.92, 1.0, 3.6 mg/kg and 7 trials on pears yielding residues of <0.6 (7) mg/kg. Because all selected data points are in the same range, the Meeting decided to combine residue results from all selected trials, both from apples and pears (STMR underlined): 0.043, <0.05, 0.11, 0.21, 0.23, 0.34, 0.40, <u><0.6</u> (30), 0.60, 0.66, 0.67, 0.73, 0.76, 0.80, 0.89, 0.92, 1.0, 3.6 mg/kg.

The Meeting agreed to withdraw the previous maximum residue level recommendation for apples and pears (1 mg/kg) and estimated a maximum residue level of 5 mg/kg, and an STMR of 0.6 mg/kg for pome fruit.

<u>Stone fruit</u>. Residue trials on peaches and plums were conducted in the USA (1997, 1998). There is no registered use in the USA.

The Meeting agreed to withdraw the previous maximum residue level recommendation of 1 mg/kg for plums (including prunes).

<u>Berries and other small fruits</u>. Residue trials on blackcurrants (1) and gooseberries (1) were conducted in the UK (1978). Diflubenzuron is not registered in the UK on gooseberries, but it is for use on blackcurrants. Of the trials conducted in the UK (1978), no trial was conducted at the UK critical GAP.

The Meeting agreed not to establish an MRL for blackcurrants and gooseberries.

<u>Brassica (cole or cabbage) vegetables, head cabbages, flowerhead cabbages</u>. Residue trials on Brussels sprouts were conducted in The Netherlands (1976) and the UK (1977, 1978). Diflubenzuron is not registered for use on Brussels sprouts in The Netherlands, but these trials could be evaluated against UK GAP. Diflubenzuron is registered in the UK for use on Brussels sprouts with SC 480 or WP 250 formulations with 2 applications at 0.1 kg ai/ha with 0.01-0.02 kg ai/hL with a PHI of 14 days. Of the trials conducted in The Netherlands and the UK, none of the trials was conducted at the critical UK GAP.

The Meeting agreed to withdraw the previous maximum residue level recommendation of 1 mg/kg for Brussels sprouts.

Residue trials on cabbage were conducted in The Netherlands (1974), the UK (1978), Germany (1975) and Brazil (1986). There is no registered use on cabbage in Brazil and Brazilian trials could not be evaluated against a GAP from another country. Diflubenzuron is not registered for use on cabbage in The Netherlands and Germany, but these trials could be evaluated against UK GAP.

Diflubenzuron is registered in the UK for use on cabbage with SC 480 or WP 250 formulations with 2 applications at 0.1 kg ai/ha with 0.01-0.02 kg ai/hL with a PHI of 14 days. Of the trials conducted in The Netherlands, Germany and the UK, residues complying with UK critical GAP are: 0.058 mg/kg for the double application on cabbage (UK 1978).

Because one trial is insufficient for the estimation of a maximum residue level, the Meeting agreed to withdraw the previous recommendation for head cabbage of 1 mg/kg.

Fruiting vegetables, other than cucurbits. Residue trials on sweet peppers and chili peppers were conducted in the USA (1997). There is no registered use in the USA, and trials could not be evaluated against another GAP.

Residue trials on tomatoes were conducted in the UK (1977, 1978) and Brazil (1989, 1991). There is no registered use in the UK, but the UK trials could be evaluated against GAP from Poland (glasshouse use): 0.2 kg ai/ha, 0.01 kg ai/hl, PHI 7 days. Assuming the UK trials were performed in a glasshouse, all were according to GAP, yielding residues of 0.075, 0.74, 0.92 mg/kg.

Diflubenzuron is registered in Brazil for use on tomatoes, but because no printed label or registration certificate is available, the trials may not be evaluated against this GAP. The trials can however be evaluated against the critical GAP for Ecuador (WP 250 formulation, at 0.12 kg ai/ha with a PHI of 14 days) or the GAP for Uruguay (WP 250 or SC 480 formulation, at 0.12 kg ai/ha with a PHI of 15 days). Of the trials conducted in Brazil, residues complying with Ecuadorian critical GAP are: 0.066 mg/kg for the single application on tomatoes (Brazil 1989).

Because results below 0.2 mg/kg are considered not valid (matrix interferences), these results are expressed as <0.2 mg/kg. Adjusted results are: <0.2 (2), 0.74, 0.92 mg/kg for applications on tomatoes.

Because four trials are insufficient for the estimation of a maximum residue level on tomatoes, the Meeting agreed to withdraw the previous recommendation (1 mg/kg).

Residue trials on mushrooms were conducted in the Netherlands (1975, 1976, 1977), Australia (1992) and in the USA (1996, 1997).

Diflubenzuron is registered in The Netherlands for use on mushrooms as a single compost or casing treatment at 10 kg ai/ha or 0.06-0.10 kg ai/hL. Of the trials conducted in The Netherlands, residues complying with Dutch critical GAP are: 0.042 - 0.062 mg/kg (Netherlands 1977). Because residue results below 0.5 mg/kg DFB are considered not valid (matrix interferences, recoveries), these results are expressed as <0.5 mg/kg resulting in <0.5 (2) mg/kg diflubenzuron for casing application.

Diflubenzuron is registered in Australia for use on mushrooms as a casing treatment at 5 g ai/bale or a single compost treatment at 10 g ai/tonne or a casing drench treatment at 10 kg ai/ha with WP250 formulations. The critical GAP is the casing treatment. Of the trials conducted in Australia, 1 of the trials was conducted at the critical Australian GAP: 1x 7.5 kg ai/ha at casing. The residue was: 0.021 mg/kg (Australia 1992).

Diflubenzuron is registered in the USA for use on mushrooms as a compost treatment at 29-49 kg ai/ha and/or as a casing treatment at 10 kg ai/ha at 0.063 kg ai/hL with SC 480 or WP250 formulations. The critical GAP is either the compost treatment or the casing treatment or a combination of both. Residues complying with the USA critical GAP are: <0.01 (5), 0.01, 0.02 (2) mg/kg for the single compost application and 0.05, 0.06, 0.07, 0.09, 0.11, 0.14, 0.21 mg/kg for the single casing application and 0.04 (2), 0.05, 0.08, 0.10 (2) mg/kg for the combined compost plus casing application (USA 1996, 1997). From the four flushes per trial the highest residue was selected. The Meeting observed that the single casing treatment and the combined compost plus casing treatment resulted in higher diflubenzuron residues than the single compost treatment. Results from the single compost treatment are therefore not used for MRL estimation. The Meeting decided to combine the other data. The selected residues from 13 trials according to USA critical GAP are: 0.04, 0.04, 0.05, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.10, 0.11, 0.14, 0.21 mg/kg.

The Meeting decided to combine all casing and combined compost plus casing trials from The Netherlands, Australia and the USA (STMR underlined). Because the LOQ of the method in the Dutch trials (>0.5 mg/kg) is too high, the values from the Dutch trials (<0.5 (2)) were not used. Residues resulting from single casing or a combined compost plus casing treatment resulted in: 0.021, 0.04 (2), 0.05 (2), 0.06, 0.07, 0.08, 0.09, 0.10 (2), 0.11, 0.14, 0.21 mg/kg.

The Meeting decided to withdraw the current recommendation for mushrooms of 0.1 mg/kg and estimated a maximum residue level of 0.3 mg/kg and an STMR of 0.075 mg/kg.

Pulses

Two residue trials on peas were conducted in the UK (1978). There is no registered use in the UK on peas. There is GAP on vegetables in Ireland but the trials did not match. The Meeting decided not to recommend an MRL for peas.

Four residue trials on soybeans were conducted in the USA (1996). Diflubenzuron is registered in the USA for use on soybeans with OF 240 or SC 240 formulations with 1-2 applications with an interval of 30 days at 0.035-0.070 kg ai/ha with a PHI of 21 days with low volume spray at 0.011-0.083 kg ai/hL or low volume spray by aeroplane at 0.075-0.25 kg ai/hL. Of the trials conducted in USA (1996), 2 trials were conducted at the critical USA GAP. Diflubenzuron residues were <0.05 (2) mg/kg in dry soybeans.

Because two trials are insufficient for the estimation of a maximum residue level on soybeans, the Meeting agreed to withdraw the previous recommendation of 0.1 mg/kg.

Cereal grains. Diflubenzuron is registered in the USA for use on rice with an SC 240 formulation by 1-2 low volume spray applications by aeroplane with an interval of 5-7 days with 0.14-0.28 kg ai/ha with 0.30-0.60 kg ai/hL and a PHI of 80 days. Residue trials on rice were conducted in the USA (1995, 1996, 1998). Storage stability of diflubenzuron and CPU in rice samples was not validated beyond a 1 month time period, because of analytical problems. In this 1 month, diflubenzuron and CPU were stable. In 6 of the 21 trials, the time that samples were stored before diflubenzuron analysis (162-388 days) exceeded the validated storage stability time by a large margin, and therefore these trials are not used for MRL estimation. In the remaining 15 trials storage time was more appropriate (9-76 days before diflubenzuron analysis) and these trials were evaluated. Eight trials were according to USA GAP, except that the applications were not done by aeroplane. Residues in rice grain were <0.01 (8) mg/kg. Another four trials were according to GAP, yielding residues in rice grain of <0.01 (4) mg/kg.

The Meeting estimated a maximum residue level of 0.01* mg/kg in rice grain, and an STMR of 0.01 mg/kg.

Tree nuts. Residue trials on walnuts, almonds and pecans were conducted in France (1999) and the USA (1988, 1998, 1999). There is no registered GAP in the USA on tree nuts and the trials could not be evaluated against another GAP.

Diflubenzuron is registered in France for use on tree nuts with SC 150 formulations with 0.01 kg ai/hL with a PHI of 28 days. Residues at GAP were <0.05 (2) mg/kg in walnut meat (France 1999).

Two trials are insufficient for the estimation of a maximum residue level for nutmeat of walnuts, almonds or pecans.

Oilseeds. Residue trials on cotton were conducted in the USA (1993, 1995). The critical USA GAP is 6 times 0.14 kg ai/ha (interval 5-14 days) with a PHI of 14 days with either (ultra) low volume spray or low volume spray by aeroplane. None of the trials were at critical GAP. Therefore the Meeting agreed to withdraw the previous maximum residue level recommendation for cotton (0.2 mg/kg).

Legume animal feed. Four residue trials on soybeans were conducted in the USA (1996). Diflubenzuron is registered in the USA for use on soybeans with OF 240 or SC 240 formulations with 1-2 applications with an interval of 30 days at 0.035-0.070 kg ai/ha with a PHI of 21 days with low volume spray at 0.011-0.083 kg ai/hL or low volume spray by aeroplane at 0.075-0.25 kg ai/hL. Residues at the critical USA GAP were 0.06-0.10 mg/kg in soybean forage (after 1 treatment) and 0.42-0.70 mg/kg in soybean hay (after 1 treatment). Because residue results below 1.0 mg/kg in soybean forage and below 3 mg/kg DFB in soybean hay are considered not valid (matrix interferences), these results are expressed as <1.0 mg/kg, or <3.0 mg/kg resulting in <1 (2) mg/kg for soybean forage and <3 (2) mg/kg in soybean hay.

Two trials are insufficient for the estimation of a maximum residue level on soybean forage and hay.

Straw, fodder and forage of cereal grains and grasses. Residue trials on rice were conducted in the USA (1995, 1996, 1998). Diflubenzuron is registered in the USA for use on rice with an SC 240 formulation by 1-2 low volume spray applications by aeroplane with an interval of 5-7 days with 0.14-0.28 kg ai/ha with 0.30-0.60 kg ai/hL and a PHI of 80 days. Nine trials were according to USA GAP, except that the applications were not done by aeroplane. Residues in rice straw were <0.01, 0.01, 0.02, 0.06 (2), 0.14, 0.25, 0.40, 0.48 mg/kg. Another six trials were according to GAP, yielding residues in rice straw of <0.01 (4), 0.02, 0.15 mg/kg. The Meeting decided to combine the datasets resulting in <0.01 (5), 0.01, 0.02 (2), 0.06 (2), 0.14, 0.15, 0.25, 0.40, 0.48 mg/kg.

The Meeting decided to recommend a maximum residue level of 0.7 mg/kg in rice straw, and an STMR of 0.02 mg/kg.

Residue trials on cotton were conducted in the USA (1993, 1995). None of the trials were at critical GAP.

Residue trials on range grass_were conducted in the USA (1991, 1992). Diflubenzuron is registered in the USA for use on pasture/rangeland with a critical GAP of 0.018 kg ai/ha with an unknown PHI with either low volume spray or low volume spray by aeroplane. Two trials with ultra low volume spray applications by aeroplane were not used, because samples from 0.035 kg ai/ha and 0.018 kg ai/ha applications are probably mislabelled. Diflubenzuron residues complying with USA GAP from low volume spray applications were: 0.65, 1.5 , 1.8, 2.0 (2), 2.3, 2.7, 3.4 mg/kg for fresh grass. Diflubenzuron residues from ultra low volume spray applications by aeroplane were: 0.37, 0.60, 0.70, 0.84, 0.88, 0.92, 2.2, 2.6 mg/kg in fresh grass and 0.28, 0.61, 0.86, 1.1, 1.2, 2.1, 2.2, 2.7 for dry grass. Results from fresh grass below 0.7 mg/kg are considered not valid (matrix interferences) and these results are expressed as <0.7 mg/kg.

The Meeting observed that residues from ultra low volume spray by aeroplane were comparable to those from low volume spray treatment, and The Meeting decided to combine both treatments. Corrected residues on fresh grass complying to USA GAP were (STMR underlined): <0.7 (3), 0.70, 0.84, 0.88, 0.92, 1.5, 1.8, 2.0 (2), 2.2, 2.3, 2.6, 2.7, 3.4 mg/kg. Diflubenzuron residues on dry grass complying to USA GAP were: 0.28, 0.61, 0.86, 1.1, 1.2, 2.1, 2.2, 2.7 mg/kg. Diflubenzuron residues in dry and fresh grass are valid in the range 1.0-4.0 mg/kg.

The Meeting estimated a highest residue level of 5 mg/kg (fresh weight) and an STMR of 1.65 mg/kg in fresh grass.

Fate of residues in storage and during processing

Fate of residues in storage

The Meeting received information on the fate of residues during storage of wheat grain. The wheat grain was spray treated post-harvest with a single 2 or 4 g ai/tonne. Diflubenzuron levels remained stable for 9 months at 1.4-1.8 mg/kg for 2 g ai/tonne and 3.2-4.4 mg/kg DFB for 4 g ai/tonne.

Fate of residues during processing

The Meeting received information on the fate of incurred residues of diflubenzuron during the processing of oranges, apples, pears, plums, mushrooms, rice, wheat and soybean.

Oranges containing 0.66 mg/kg diflubenzuron eq radiolabelled residues were subjected to small scale processing into orange oil. In the oranges 95% TRR was parent while 5% TRR was unextracted radiolabel. In the orange oil only parent was found. Total diflubenzuron residues were concentrated in the citrus oil, the calculated processing factor for the parent compound is 68. When corrected for weight fractions, the % transference is 9.8%.

Apples, grown in the USA in 1983 and treated with diflubenzuron, were subjected to industrial processing into canned apple sauce, wet and dry pomace, pasteurised apple juice and apple butter. Diflubenzuron was concentrated in wet and dry apple pomace, the calculated processing factors were 3.6, 4.6, 4.7(2), 5.2, 5.6, 5.9, 6.1 (average 5.0) for wet apple pomace and 9.3, 12 (2), 13, 14 (2), 16, 17, (average 13) for dry apple pomace. Diflubenzuron was diluted in canned apple sauce and pasteurized apple juice: residues were below the LOQ (0.05 mg/kg)and therefore their processing factors were in each trial calculated to be identical: <0.058, <0.062, <0.078, <0.098, <0.14, <0.15, <0.16, <0.28 (average <0.12). The LOQ for apple butter was too high (0.5 mg/kg) to draw conclusions on dilution or concentration of residues.

In another trial, apples, grown in Germany (1993) and treated with diflubenzuron, were subjected to household processing into raw apple juice and wet apple pomace. Diflubenzuron was concentrated in wet apple pomace, the calculated processing factors were 2.1, 1.3, 2.4, 1.1 (average 1.7). Diflubenzuron was diluted in raw apple juice, the calculated processing factors were 0.14, 0.086, 0,.15, 0.061 (average 0.11). The % transference was 3.0%-7.5% for apple juice and 55%-120% for wet apple pomace. In this study there were residue losses, because the sum of % transference in apple juice and apple pomace is in some cases lower than 80%.

The Meeting decided not to combine the processing factors from the USA and German trials and to use the average processing factor of 5.0 for wet apple pomace in the calculation of the dietary burden for livestock. From this processing factor and the STMR for apples (0.6 mg/kg) the Meeting estimated an $STMR_P$ for wet apple pomace of 3 mg/kg. From the average processing factor of <0.12 for pasteurized apple juice and the STMR for apples (0.6 mg/kg) the Meeting estimated an $STMR_P$ of 0.072 for apple juice.

Pears, grown in the USA (1983), were subjected to industrial processing into canned pears. Diflubenzuron was diluted in canned pears: residues were below the LOQ (0.05 mg/kg) and accurate processing factors could not be calculated.

Plums, grown in the USA (1998), were dried for 18 hours. Diflubenzuron was not concentrated during processing to prunes: the processing factor for diflubenzuron for the preparation of prunes is 1. When corrected for weight fractions, the % transference is 32%; it is unclear where the remaining residues went. Because of low recoveries, results are considered not valid.

Mushrooms treated with ^{14}C-DFB in compost or casing were harvested in 3 flushes. The combined flushes were canned. During canning more than 70% of the radioactivity present in the mushrooms moved into the canning liquid. The main part of the residue in the canning liquid consisted of DFBA (>100%); the residue in canned mushrooms consisted of parent (1.8%-14%), DFBA (46%-72%) and unextracted radiolabel (20%-39%). Processing factors for canned mushrooms were 0.43 and 2.5 for diflubenzuron for casing and compost treatment, respectively.

Mature pods from soybean plants were harvested 62 days after treatment with ^{14}C-DFB. Mature pods were separated in seeds and hulls and oil was extracted on laboratory scale. On the treated pods, >99% of the recovered radioactivity was found in the hulls and less than 0.2% was found in the seeds. In the soybean oil ^{14}C residues were near the LOQ (0.01 mg/kg oil) and there was no significant difference between oil from treated or from untreated pods: 0.014 and <0.01-0.012 mg/kg, respectively. From this study, The Meeting concluded that diflubenzuron does not accumulate in soybean oil.

In four trials (1995, 1996, USA), rice was harvested 82-115 days after a single spray or a single spray by aeroplane with diflubenzuron at 2.2 kg ai/ha (8x exaggerated dose for USA). Rice grain was processed into polished rice, hulls and bran. Diflubenzuron, CPU and PCA were analysed. In one trial only, residue was detected in the rice grain itself. From this trial, the calculated processing factors for diflubenzuron were: hulls 0.39, bran 0.14, polished rice <0.018. When corrected for weight fractions, the % transference is hulls 0.08%, bran 0.03% and polished rice <0.01%. Storage stability of diflubenzuron and CPU was not validated beyond a 1 month time period, because of analytical problems. In the rice processing trial described above, diflubenzuron was measured after 351-388 days, and CPU after 499-542 days. Since the storage time of the samples exceeded the validated storage stability time by a large margin, the results of this processing trial are considered unreliable.

Wheat grain, treated post-harvest with 2 g ai/tonne, was stored for 4 months at ambient temperatures. Wheat grain was processed into sieved wheat and bran. The sieved wheat was further processed into Buehler flour, first reduction flour, wholemeal flour, white bread and whole meal bread. Diflubenzuron was concentrated in bran: processing factors 1.9, 2.3(2). Diflubenzuron was diluted in flour and bread: processing factors 0.32, 0.34, 0.35 in first reduction flour, 0.15, 0.17, 0.18 in Buehler flour, 0.20, 0.22, 0.23 in white bread, 0.62, 0.72, 0.74 in whole meal flour and 0.40, 0.47, 0.49 in whole meal bread. Transferences could not be calculated.

Farm animal dietary burden

The Meeting estimated the dietary burden of diflubenzuron residues in farm animals from the diets listed in Appendix IX of the FAO Manual (FAO, 2002). Calculation from the HR values provides the concentrations in feed suitable for estimating MRLs for animal commodities, while calculation from the STMR values for feed is suitable for estimating STMR values for animal commodities. In the case of processed commodities, the STMR-P value is used for both intake calculations.

Maximum farm animal dietary burden estimation

Commodity	Residue, mg/kg for dietary intake	Basis	% DM	Residue	Group	Feed allocation to total diets (%)				Residue contribution of feeds (mg/kg)			
				mg/kg dw		Beef cattle	Dairy cows	Poultry	Swine	Beef cattle	Dairy cows	Poultry	Swine
Apple, wet pomace	3	STMR_P	40	7.5	AB	40	20			3	1.5		
Grass, forage	5	MRL	25	20	AF	60	60			12.00	12.00		

				Residue	Group	Feed allocation to total diets (%)				Residue contribution of feeds (mg/kg)			
Rice, grain	0.01	MRL	88	0.01	GC		10	60	65		0.001	0.01	0.01
Rice, straw	0.7		90	0.78	AS		10				0.078		
TOTAL						100%	100%	60%	65%	15.0	13.58	0.01	0.01

Mean farm animal dietary burden estimation

Commodity	Residue, mg/kg for dietary intake	Basis	% DM	Residue mg/kg dw	Group	Feed allocation to total diets (%)				Residue contribution of feeds (mg/kg)			
						Beef cattle	Dairy cows	Poultry	Swine	Beef cattle	Dairy cows	Poultry	Swine
Apple, wet pomace	3	STMR$_P$	40	7.5	AB	40	20			3.0	1.5		
Grass, forage	1.65	STMR	25	6.6	AF	60	60			3.96	3.96		
Rice, grain	0.01	STMR	88	0.01	GC		10	60	65		0.001	0.01	0.01
Rice, straw	0.02	STMR	90	0.02	AS		10				0.002		
TOTAL						100%	100%	60%	65%	6.96	5.46	0.01	0.01

Direct treatment of farm animals

Four studies on direct animal treatments are available for sheep. In all studies, only parent compound was analysed.

In the first trial male Merino sheep were treated within 24 hours after shearing with a dose corresponding to 1.5-2x the standard dose for pour-on application in Australia. The liver, kidney and muscle tissues had levels below the LOQ (<0.02 mg/kg) at all post-treatment days, except 1 liver sample at day 1 after treatment (0.02 mg/kg). Residues were found at random in fat day 1 to day 21 post-treatment (<0.02-0.05 mg/kg), with the greatest persistence being in the peri-renal and lumbar fat. Diflubenzuron residues were at levels below the LOQ (<0.02 mg/kg) after 21 days for the peri-renal fat and after 42 days (= withdrawal period on the label) for the pre-femoral fat and lumbar fat. After 14 days, one animal gave anomalous high residues in fat (0.23-0.50 mg/kg) when compared to the rest of the animals in that group (0.03 mg/kg maximum). Low residues were also found in the liver (0.03 mg/kg) and muscle (0.02 mg/kg) of this animal.

In a trial that matched Australian label instructions for pour-on application, 5 month old Merino lambs were treated. Only fat was analysed. Residues were found randomly in fat samples from 1-42 days post-treatment (<0.02-0.13 mg/kg). After 42 days (= withdrawal period on the label) residues were found in one pre-femoral fat sample (0.04 mg/kg) and one lumbar fat sample (0.04 mg/kg) from different animals.

In a trial that matched Australian label instructions for plunge dip, ewes plunged and swum for 3 min in solution until total saturation of the fleece was obtained. Animals were slaughtered at 15 hours and 7 days post dipping (there is no waiting period on the label). No residues were found in liver and kidney (<0.03 mg/kg). Other tissues were not analysed.

In the UK, sheep were treated with diflubenzuron by pour-on application. There is no label in the UK, therefore the trial was evaluated against the Australian label. The instructions on the label were not entirely matched as sheep were shorn 7 days before treatment and the Australian label states that the product should be used on sheep with 6 weeks – 6 months wool growth. Fat contained the highest concentration of residues (max. 0.28 mg/kg) 3 days after treatment, followed by muscle (max.

0.17 mg/kg). Residues in fat and muscle declined below the LOQ (0.05 mg/kg) 10 days after treatment. Residues in liver and kidney were below the LOQ (0.05 mg/kg) at all time points.

Based on all trials above, and taking into consideration the waiting periods on the labels, the Meeting estimated a maximum residue level of 0.05 mg/kg for diflubenzuron residues in sheep meat (fat) and 0.05 mg/kg sheep offal.

The STMR concept is designed for use in supervised field trials on crops to obtain the typical residue value when a pesticide is used according to maximum GAP. The method is not directly applicable to a trial of single direct treatment of animals. However, the Meeting agreed that a typical residue value for a pesticide used directly on animals (at maximum label conditions) would be useful in estimating long-term dietary intake. The Meeting estimated a typical concentration of diflubenzuron residues (from direct use at maximum label conditions) of 0.05 mg/kg in sheep meat and sheep offal.

Farm animal feeding studies

Animal feeding studies are available for beef cattle, dairy cows, sheep and chickens. In all animal feeding studies, only parent compound was analysed.

One dairy cow was fed 1 mg/kg bw diflubenzuron per day for 119 days. Assuming a bodyweight of 550 kg and a dry feed intake of 20 kg/day, the daily intake is estimated at 28 mg/kg diflubenzuron in feed. In omental fat a residue of 0.10 mg/kg was measured. In all other samples (renal fat, diaphragmatic fat, subcutaneous fat, muscle, kidney, liver) the residue level was below the reported LOQ (0.1 mg/kg). Another dairy cow was fed with increasing levels from 1-8 mg/kg bw diflubenzuron per day for 2 week periods and after 56 days the rate was increased to 16 mg/kg bw diflubenzuron per day for 94 days. Assuming a bodyweight of 550 kg and a dry feed intake of 20 kg/day, the daily intake is estimated at 28-220 mg/kg diflubenzuron in feed for the first 55 days and 440 mg/kg diflubenzuron in feed for the remaining days. In milk no residue was found during the 1-8 mg/kg bw feeding period, but 0.02 mg/kg DFB was found during the 16 mg/kg bw feeding period (LOQ 0.02 mg/kg). In fat a residue of 0.25 mg/kg was found, and in liver a residue of 0.13 mg/kg. In all other samples (muscle, kidney) the residue was below the LOQ (0.1 mg/kg).

One Holstein bull calf was fed 2.8 mg/kg bw diflubenzuron per day from 3 days of age until slaughter at 146 days of age. Three Holstein bull calves were fed 2.8 mg/kg bw diflubenzuron per day from 3-208 days of age and thereafter with 1.0 mg/kg bw diflubenzuron per day until slaughter (at 349, 569, 571 days). Because of increasing weights and unknown feed intakes, the daily feed intake cannot be calculated but is certainly not constant. The first bull calf had 0.08 mg/kg diflubenzuron in the renal fat, 0.04 mg/kg in the omental and subcutaneous fat, 0.02 mg/kg in the liver and kidney and <0.02 mg/kg in muscle. In the tissues from the other three calves analysed, diflubenzuron was not found (reported LOQ 0.02 mg/kg).

Three bulls and three cows were fed 0.2 mg/kg bw dilubenzuron per day for 28 days (feed-through application). Taking the mean body weight of 319 kg and assuming a feed intake of 15 kg for beef cattle, the daily intake is 4.3 mg/kg diflubenzuron in feed. In one liver a residue of 0.06 mg/kg was measured. In all other samples (muscle, liver, fat and kidney) the residue level was below the LOQ (0.05 mg/kg). In the milk of six lactating cows fed at the same dose rate (feed-through application) no residues were found 3, 7, 14, 21 and 28 days after treatment (LOQ = 0.01 mg/kg).

In sheep fed with 100 mg/kg diflubenzuron in feed for 1-9 months, a maximum residue level of 1.7 mg/kg in fat, 0.58 mg/kg in liver, 0.33 mg/kg in kidney and 0.26 mg/kg in muscle was observed during the treatment period. After treatment had stopped, the residue level decreased to levels below 0.05 mg/kg (reported LOQ) in one week for muscle and in four weeks for liver and kidney. However, in fat a residue of 0.20 mg/kg was still present four weeks after treatment. In the treatment period, a maximum residue level of 0.44 mg/kg was found in milk.

Chickens (8 white, 8 brown) were fed diflubenzuron at a level of 10 mg/kg feed for 15 weeks. At all dosage levels, white eggs contained more residue than brown eggs: the mean residue was 0.38 mg/kg for brown eggs and 0.53 mg/kg for white eggs. The same was observed in the tissues: the mean residue level in white chicken liver was 0.45 mg/kg while it was 0.12 mg/kg in brown chicken liver and the mean residue level in white chicken fat was 1.8 mg/kg while it was 1.2 mg/kg DFB in brown chicken fat. In muscle, residue levels were below the reported LOQ (0.1 mg/kg).

Chickens were fed 2.5 or 250 mg/kg diflubenzuron in the feed for 98 days. The higher dosage resulted in tissue residue levels that were 7 times higher than that of the lower dosage. At the low dosage, the highest residue in fat was 6.3 mg/kg, in breast muscle + skin 0.31 mg/kg, leg muscle 0.41 mg/kg and liver 0.70 mg/kg. At the high dosage, the highest residue in fat was 56 mg/kg DFB, breast muscle + skin 2.9 mg/kg, leg muscle 2.8 mg/kg and liver 3.5 mg/kg.

Chickens were fed diflubenzuron at a level of 10 mg/kg feed for 28 days (feed-through application). White eggs contained more residue than brown eggs: the residue was 0.48-0.65 mg/kg for white eggs and 0.29-0.35 mg/kg for brown eggs. The same was observed in the tissues: the residue level in white chickens was 2.3-0.47-0.17-0.14 mg/kg, while it was 1.4-0.13-0.060-0.065 mg/kg in brown chickens for fat, liver, kidney and muscle, respectively.

Animal commodity maximum residue levels

As the maximum dietary burdens of beef and dairy cattle were 15.0 and 13.6 mg/kg, respectively, the concentrations of residues in tissues and milk were taken from the first dairy cow feeding study. This is an old feeding study which was not conducted according to current standards but which can be used because of the low animal dietary burden. When fed with increasing levels of about 28-220 mg/kg diflubenzuron in feed for two week periods, no residue was found in milk (<0.02 mg/kg). After feeding a cow 28 mg/kg diflubenzuron in feed for 119 days, no residues were found in muscle, kidney and liver (<0.1 mg/kg). In fat a residue of 0.1 mg/kg was measured.

Since the estimated dietary burden is lower than the feeding level in the study where no residue was found in milk, muscle, liver and kidney, and a residue at the LOQ was found in fat, the Meeting estimated a maximum residue level of 0.02* mg/kg in milk and a maximum residue level of 0.1* mg/kg in edible offal. For meat (fat) a maximum residue level of 0.1 mg/kg was estimated.

From the direct animal treatment studies the Meeting estimated a maximum residue level of 0.05 mg/kg for diflubenzuron residues in sheep meat (fat) and 0.05 mg/kg sheep offal. The highest value from either direct treatment or animal feeding is observed for estimation of maximum residue levels.

In conclusion, the Meeting replaced the previous maximum residue level recommendations for milks (0.05* mg/kg), meat (from mammals other than marine mammals; 0.05* mg/kg) and edible offal (mammalian; 0.05* mg/kg) by recommendations for milks of 0.02* (F) mg/kg, meat (fat) of 0.1 mg/kg and edible offal of 0.1* mg/kg. The Meeting estimated STMRs for milks of 0.02 mg/kg, and for meat and edible offal of 0.1 mg/kg.

As the maximum dietary burden for poultry is only 0.01 mg/kg feed, residues in meat and eggs are not to be expected. Since the residue is now defined as fat-soluble, the Meeting replaced the previous maximum residue level recommendation for poultry meat (0.05* mg/kg) by a recommendation for poultry meat of 0.05* (fat) mg/kg. The Meeting agreed to maintain the current recommendation of 0.05* mg/kg for eggs. The Meeting estimated STMRs for poultry meat and eggs of 0.05 mg/kg.

FURTHER WORK OR INFORMATION

Desirable

1. A ruminant feeding study according to modern standards.
2. A storage stability study in rice samples, going on for as long as 400 days for diflubenzuron analysis and 500 days for CPU analysis.

RECOMMENDATIONS

On the basis of the data from supervised trials the Meeting concluded that the residue levels listed below are suitable for establishing maximum residue limits and for IEDI assessment.

Definition of the residue (for compliance with MRLs and for estimation of dietary intake): *diflubenzuron. The definition applies to plant and animal commodities.*

The residue is fat-soluble.

Commodity		MRL, mg/kg		STMR, mg/kg	$STMR_P$, mg/kg
CCN	Name	New	Previous		
FP 0226	Apple	W	1		
JF 0226	Apple juice	-	-		0.072
VB 0402	Brussels sprouts	W	1		
VB 0041	Cabbages, Head	W	1		
FC 0001	Citrus fruits	0.5	1	0.26	
SO 0691	Cotton seed	W	0.2		
MO 0105	Edible offal (mammalian)	0.1*	0.05*	0.1	
PE 0112	Eggs	0.05*	0.05*	0.05	
MM 0095	Meat (from mammals other than marine mammals)	0.1 (fat)	0.05*	0.1 (fat)	
ML 0106	Milks	0.02*F	0.05*	0.02	
VO 0450	Mushrooms	0.3	0.1	0.075	
FP 0230	Pear	W	1		
FS 0014	Plums (including Prunes)	W	1		
FP 0009	Pome fruit	5	-	0.6	
PM 0110	Poultry meat	0.05* (fat)	0.05*	0.05 (fat)	
GC 0649	Rice	0.01*	-	0.01	
AS 0649	Rice straw and fodder, dry	0.7		0.02	
VD 0541	Soya bean (dry)	W	0.1		
VO 0448	Tomato	W	1		

DIETARY RISK ASSESSMENT

Chronic intake

The International Estimated Daily Intakes of diflubenzuron, based on the STMRs estimated for 9 commodities, for the five GEMS/Food regional diets were 1-6% of the ADI (Annex 3). The Meeting concluded that the long-term intake of residues of diflubenzuron resulting from its uses that have been considered by JMPR is unlikely to present a public health concern.

Short-term intake

The WHO panel of JMPR 2001 decided that an acute RfD is unnecessary and therefore the Meeting concluded that the short-term intake of diflubenzuron residues is unlikely to present a public health concern.

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