PYFLUBUMIDE (314)

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EXPLANATION

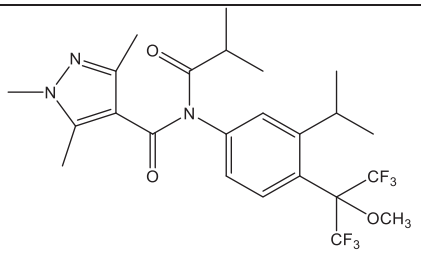
Pyflubumide, (3'-isobutyl-N-isobutyryl-1,3,5-trimethyl-4'-[2,2,2-trifluoro- 1-methoxy- 1-(trifluoro-methyl) ethyl] pyrazole-4-carboxanilide) (IUPAC name) is a new pyrazolecarboxamide acaricide used for control of mites, such as species belonging to the Tetranychus and Panonychus genera. It acts through the inhibition of the mitochondrial electron transport system complex II (succinic dehydrogenase complex).

Pyflubumide was first registered in Japan in 2015 for use on tea, fruits and vegetable crops as well as flowers and ornamental plants.

Pyflubumide was scheduled scheduled by the Forty-eighth Session of the CCPR in 2016 for toxicological and residue evaluation by the current JMPR as a new compound. No specification has been established by the Joint FAO/WHO Meeting on Pesticide Specifications for pyflubumide.

The Meeting received information on identity, physical and chemical properties, metabolism and environmental fate, residue analysis and storage stability, use pattern, supervised trials on apple and tea, and processing studies on apple and tea.

IDENTITY

ISO common name:	Pyflubumide
Chemical name	
IUPAC:	3'-isobutyl-*N*-isobutyryl-1,3,5-trimethyl-4'-[2,2,2-trifluoro-1-methoxy-1-(trifluoro-methyl)ethyl] pyrazole-4-carboxanilide
CAS:	1,3,5-trimethyl-*N*-(2-methyl-1-oxopropyl)-*N*-[3-(2-methylpropyl)-4-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)ethyl]phenyl]-1*H*-pyrazole-4-carboxamide
CAS Registry No.:	926914-55-8
CIPAC No.:	N/A
Structural formula:	
Molecular formula:	$C_{25}H_{31}F_6N_3O_3$
Molecular mass:	535.52 g/mol

PHYSICAL AND CHEMICAL PROPERTIES

Pyflubumide Property	Results	Reference
Appearance	White (N9.5/90.0%R) powder (20 °C) (purity 99.1%)	Brekelmans Ir. M.J.C., 2011 (PC-32008)
Odour	No characteristic odour (20 °C) (purity 99.1%)	Brekelmans Ir. M.J.C., 2011 (PC-32008)
Relative density	Average density: 1.277 g/cm^3 (20 °C) Specific density: d^{20}_{20}=1.279 (20 °C) (purity 99.1%)	Hori K, 2009 (PC032005)

Pyflubumide Property	Results	Reference
Melting point	86 °C (359K) (purity 99.1%)	Brekelmans Ir. M.J.C., 2011 (PC-32008)
Boiling point	Decomposition prior to boiling at 225 °C (498K) (purity 99.1%)	Brekelmans Ir. M.J.C., 2011 (PC-32008)
Thermal stability	Stable; no reaction and/or decomposition was observed below 150 °C under nitrogen and air (purity 99.1%)	Brekelmans Ir. M.J.C., 2011 (PC-32008)
Vapour pressure	1.9×10^{-6} Pa at 20 °C 4.5×10^{-6} Pa at 25 °C (Purity 99.1%)	Brekelmans Ir. M.J.C., 2011 (PC-32008)
Henry's law constant	3.77×10^{-3} Pa·m^3·mol^{-1} (20 °C)(calculated from the water solubility and vapour pressure) (purity 99.1%)	-
Solubility in water	0.27 mg/L at 20 °C and pH 6.88 (purity 99.1%)	Masaki T., 2010 (PC-32010)
Solubility in organic solvents at 20 ± 0.5 °C	Heptane, 22.8 g/L Xylene, ≥ 250 g/L 1,2-Dichloroethane, ≥ 250 g/L Acetone, ≥ 250 g/L Methanol, ≥ 250 g/L Ethyl acetate, > 250 g/L (purity 99.1%)	Hori K., 2009 (PC-32006)
Octanol/water partition coefficient (Log P_{ow})	5.34 at pH 7.35 at 25 °C (purity 99.1%)	Masaki T., 2010 (PC-32009)
Hydrolysis in sterile buffer in the dark (average of phenyl and pyrazole label)	Half-life at 20 °C pH 4, 41.7 days pH 7, 37.4 days pH 9, 15.1 days Half-life at 25 °C pH 4, 32.4 days pH 7, 27.9 days pH 9, 6.6 days (purity of phenyl label 99.2%; and pyrazole label 99.1%)	Masaki T., 2012 (E-32001)
Photolysis in sterile water under artificial light	Half-life in sterilized natural river water at 25 ± 1 °C 0.9 day under Xenon arc artificial irradiation (purity of phenyl label, 98%; and pyrazole label, 98%)	Masaki T., 2011 (E-32003)
Dissociation constant in water	pKa, 4.31 (basic group) (purity 99.1%)	Brekelmans Ir. M.J.C., 2011 (PC-32008)
Stability of TGAI	72 months when stored at room temperature and protected from light (purity 97.7%)	Oshima T., 2013 (PC-32033)
pH of TGAI	6.9 at 25 °C (purity 95.8%)	Kawashima H., 2018 (PC-32035)

P-NH metabolite

Property	Results	Reference
Vapour pressure	$< 1.3 \times 10^{-8}$ Pa at 20 °C $< 6.5 \times 10^{-8}$ Pa at 25 °C (purity 99.5%)	Brekelmans Ir. M.J.C., 2011 (PC-32013)
Solubility in water	12.32 ± 0.78 µg/L at 20 ± 1 °C (purity 99.5%)	Ihara T., 2009 (PC-32014)
Octanol/water partition coefficient (Log P_{ow})	5.02 at pH 6.26 at 25 °C (purity 99.5%)	Masaki T., 2012 (PC-32015)

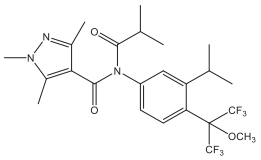
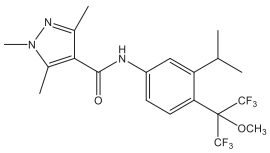
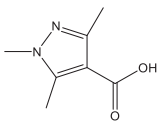
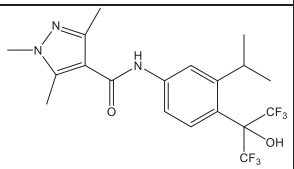
Formulations

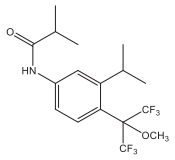
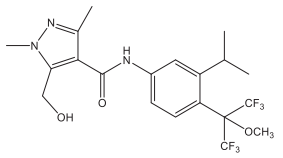
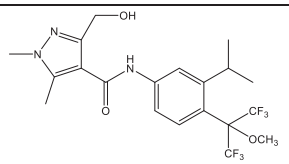
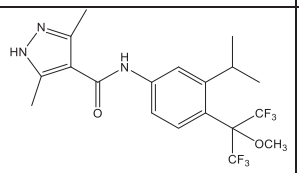
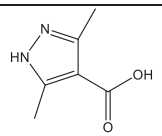
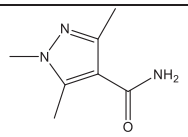
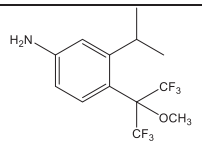
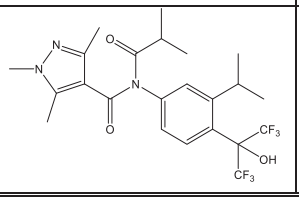
Pyflubumide is formulated singly or in combination with 5% or 10% fenpyroximate, as an SC containing 200 g ai/L.

METABOLISM AND ENVIRONMENTAL FATE

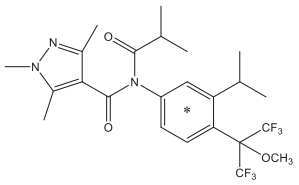
The following table links code numbers and structures of the compounds appearing in the various metabolism and environmental fate studies.

Table 1 Structure of compounds appearing in metabolism and environmental fate studies.

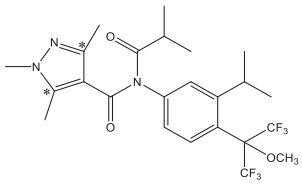
Compound Name/Code (MW)	IUPAC name	Structure	Found in study on:
Pyflubumide/ NNI-0711 (535.52)	3'-isobutyl-*N*-isobutyryl-1,3,5-trimethyl-4'-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl) ethyl]pyrazole-4-carboxanilide		Apple Eggplant Spinach Rat excreta Soil Hydrolysis Photolysis
P-NH/ NNI-0711-NH (465.43)	3'-isobutyl-1,3,5-trimethyl-4´-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)ethyl]pyrazole-4-carboxanilide		Apple Eggplant Spinach Rat Soil Hydrolysis Photolysis
P-acid/ NNI-0711-acid (154.17)	1,3,5-trimethylpyrazole-4-carboxylic acid		Eggplant Spinach Rat excreta Hydrolysis Photolysis
P-NH-RfOH/ NNI-0711-NH-RfOH (451.41)	3'-isobutyl-1,3,5-trimethyl-4´-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]pyrazole-4-carboxanilide		Eggplant Rat blood Soil

Compound Name/Code (MW)	IUPAC name	Structure	Found in study on:
P-aniline-isobutyryl/ NNI-0711-aniline-isobutyryl (385.34)	3'-isobutyl-4-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)- ethyl]phenyl]isobutylanilide		Rat GI tract Eggplant Soil Hydrolysis Photolysis
P-NH-5-CH_2OH/ NNI-0711-NH-5-CH_2OH (481.43)	5'-hydroxymethyl)-3'-isobutyl-1,3-dimethyl-4´-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)ethyl] pyrazole-4- carboxanilide		Eggplant Soil
P-NH-3-CH_2OH/ NNI-0711-NH-3-CH_2OH (481.43)	3-(hydroxymethyl)-3'-isobutyl-1,5-dimethyl-4'-[2,2,2-trifluoro-methoxy-1-(trifuluoromethyl) ethylpyrazole-4-caroxanilide		Soil
P-NH-1-H/ NNI-0711-NH-1-H (451.40)	3'-isobutyl-3,5-dimethyl-4´-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)ethyl]pyrazole-4-carboxanilide		Rat blood Photolysis
P-acid-1-H/ NNI-0711-acid-1-H (140.14)	3,5-dimethylpyrazole-4-carboxylic acid		Rat excreta Photolysis
P-amide/ NNI-0711-amide (153.18)	1,3,5-trimethylpyrazole-4-carboxamide		Photolysis
P-aniline/ NNI-0711-aniline (315.25)	3-isobutyl-4-[2,2,2-trifuluoro-1-methoxy-(trifuluoromethyl)ethyl] aniline		Photolysis
Pyflubumide-RfOH NNI-0711-RfOH	3'-Isobutyl-N-isobutyryl-1,3,5-trimethyl-4'-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl] pyrazol-4-carboxanilide		Rat

The Meeting received information on plant metabolism of pyflubumide, its environmental fate in soil, and hydrolysis and photochemical degradation in water. The fate and behaviour of pyflubumide in plants, and soil, and hydrolysis/photolysis were investigated using the radiolabelled pyflubumide with ^{14}C in the phenyl ring (uniformly labelled) or at positions 3 and 5 of the pyrazole ring as shown in Figure 1.



[Phenyl-UL-^{14}C] Pyflubumide
abbreviated as [phenyl-^{14}C]- pyflubumide or phenyl-label



[Pyrazole-3(5)-^{14}C] Pyflubumide
(labelled at potion 3 or 5 of the pyrazole ring)
Abbreviated as [pyrazole-^{14}C]-pyflubumide or pyrazole-label

Figure 1 Radio-labelled test materials used in the metabolism and environmental fate studies

PLANT METABOLISM

The Meeting received information on metabolism of radio-labelled pyflubumide labelled with ^{14}C in the phenyl ring or at positions 3 and 5 of the pyrazole ring in apple, eggplant and spinach to cover proposed uses of pyflubumide. In the following texts, TRR is expressed in mg-pyflubumide equivalents/kg.

Apple (Dohn R., et al., 2012; R-32001)

The metabolism of pyflubumide was studied on nine-year-old apple trees (variety Fuji) grown in an orchard (three plots with one apple tree in each plot) in California. Each apple tree was pruned to a projected area of 1.5 m^2. The soil type in the orchard was loamy sand with a pH of 8.2 and an organic matter content of 0.4%.

Both the [phenyl-^{14}C]- and [pyrazole-^{14}C]-pyflubumide were formulated as a 20% SC and applied once as a foliar spray to leaves and fruits according to the normal agricultural practice at rates of 357 g ai/ha (phenyl label) or 349 g ai/ha (pyrazole label) to one apple tree while the target was 400 g ai/ha (concentration, ca. 10 g ai/hL). Plastic sheeting was erected around the plot and covered the top and ground of the plot to block wind and removed after the application.

Fruits and leaves were collected 0, 7, 14, 28 and 51 days after the treatment (end of August to middle of October). The PHI on the label for apple is 1 day. All samples were delivered to the laboratory on the day of collection. The samples of fruits and leaves were washed two times with acetone by immersion and the washes were combined for each crop fraction and stored at approximately -20 °C. The washed fruit and leaf samples were homogenized in dry ice using food processors. Prior to further analysis the dry ice was allowed to sublime in the freezer.

Samples (approximately 250 mg each, five replicates from each primary sample) of the homogenized apple fractions from treated and control trees were analysed by combustion/liquid scintillation counting (LSC). A portion of each apple fraction (approximately 50 g of fruit or 25 g of leaves) was extracted three times, first with 75 mL of acetone, second with 75 mL of acetone:H_2O (1:1, v/v) and third with 75 mL acetone:1M HCl (1:1 v/v), using a probe type homogenizer (for 2 min), and then by a wrist action shaker (for 20 min). The homogenates were centrifuged to separate solids and extracts. The extracts were combined and analysed by LSC. Aliquots of the combined extracts were concentrated by rotary evaporation for HPLC analysis. The radioactivity remaining in the post-extraction solids (PES) were quantified by combustion/LSC analysis.

Portions of PES from the four leaf samples collected 28 and 51 days after the treatment with phenyl or pyrazole label were treated in sequence with 1 M HCl (50 °C for 4 hours), 6 M HCl (50 °C for 4 hours), 1 M KOH (50 °C for 4 hours) and then 24% (w/v) KOH (25 °C for 16.5 hours). After each treatment, the suspension was separated in to solid for further treatment or combustion analysis and extract which was analysed for radioactivity by LSC.

Aliquots of acetone surface wash and the first extract (each equivalent to 1 g of leaf) from the day 51 leaf sample of pyrazole label treatment were combined and reduced to dryness by rotary evaporation. Dried sample was dissolved in tetrahydrofuran and incubated with beta-glucosidase in 0.1 M sodium acetate buffer at pH 5.0 at about 37 °C.

Metabolites were identified by one or two-dimensional TLC and HPLC-UV (gradient with 0.1% acetic acid in water and acetonitrile containing 0.1% acetic acid; detection at 260 nm).

The total radioactive residues (TRR) in the apple fruit and leaf samples collected after treatment with phenyl- or pyrazole-labelled pyflubumide are shown in Table 2.

One application of either of the radioactive labelled pyflubumide compounds resulted in similar TRR in fruits or leaves showing decrease of radioactive residues over time (up to 51 days). The TRR in fruits were low at 0.049–0.19 mg eq/kg and those in leaves were much higher at 5.1–17.4 mg eq/kg.

Table 2 Total radioactive residues in the fruit and leaf samples from apple trees treated with radioactive pyflubumide at a target rate of 400 g ai/ha.

Label	TRR (mg pyflubumide equivalents/kg)				
	DAT 0	DAT 7	DAT 14	DAT 28	DAT 51
Fruit					
Phenyl-label	0.188	0.090	0.108	0.068	0.058
Pyrazole-label	(0.161)[a]	0.096	0.086	0.049	0.068
Leaf					
Phenyl-label	17.1	11.5	8.5	6.6	5.4
Pyrazole-label	17.3	12.3	9.9	6.8	5.1

[a] Sum of radioactivity in washes and that in washed fruit by combustion.

The distribution of radioactivity in the acetone surface wash, combined extract of acetone, acetone/water, and acetone/HCl, and PES of fruit and leaf samples is shown in Table 3.

The distribution and tendency of radioactivity in fruits and leaves were similar regardless of the position of the ^{14}C label. The radioactive residues as well as the percentage of TRR in acetone surface wash decreased while those in the extracts (acetone and acetone mixtures) in PES increased gradually over time. The largest portion of the radioactivity was recovered in surface wash.

In the acetone surface wash of fruit, the radioactive residues were recovered in the surface wash on DAT 0 at 94–98% TRR and 0.16–0.18 mg eq/kg, decreased on DAT 7 to 77–80% TRR and 0.069–0.077 mg eq/kg and further decreased to 59–65% TRR and 0.034–0.044 mg eq/kg on DAT 51. The acetone and acetone mixtures extracted 6.4% TRR and 0.012 mg eq/kg on DAT 0, increased to 17–19% TRR (0.016–0.017 mg eq/kg) on DAT 7, and to 27–28% TRR and 0.016–0.018 mg eq/kg, on DAT 51. The radioactivity remained unextracted increased to 8.8–14% TRR (0.006–0.008 mg eq/kg) on DAT 51.

In apple leaves, on DAT 0, most of the radioactivity (94–98% TRR, 16–17 mg eq/kg) was recovered in the surface wash, on DAT 7, 86–92% TRR (9.9–11 mg eq/kg), and on DAT 51, 54–57% TRR (2.9 mg eq/kg). The acetone and acetone mixtures extracted 2.3–6.1%TRR (0.4–1.0 mg eq/kg) on DAT 0, increasing to 6.4–11% TRR (0.77–1.3 mg eq/kg) on DAT 7 and 34–35% TRR (1.8 mg eq/kg) on DAT 51. The radioactivity remained unextracted increased from only 0.1–0.2% TRR (0.0021–0.038 mg eq/kg) on DAT 0 to 8.9–13% TRR (0.45–0.69 mg eq/kg) on DAT 51.

Table 3 Radioactive residues in fractions of the fruit and leaf samples from apple trees treated with radioactive pyflubumide at a nominal rate of 400 g ai/ha.

Fraction	DAT 0		DAT 7		DAT 14		DAT 28		DAT 51	
	mg/kg [a]	% TRR	mg/kg [a]	% TRR	mg/kg [a]	% TRR	mg/kg [a]	% TRR	mg/kg [a]	% TRR
Fruit (Phenyl-label)										
Acetone surface wash	0.176	93.6	0.069	76.7	0.073	67.6	0.034	50.0	0.034	58.6
Extract (acetone, acetone/water, & acetone/HCl)	0.012	6.4	0.017	18.9	0.028	25.9	0.025	36.8	0.016	27.6
PES	0.000	0	0.004	4.4	0.007	6.5	0.009	13.2	0.008	13.8
Total	0.188	100	0.090	100	0.108	100	0.068	100	0.058	100
Fruit (Pyrazole-label)										
Acetone surface wash	0.157	97.5	0.077	80.2	0.065	75.6	0.028	57.1	0.044	64.7
Extract (acetone, acetone/water, & acetone/HCl)	[b/]	-	0.016	16.7	0.018	20.9	0.017	34.7	0.018	26.5
PES[b/]	-	-	0.003	3.1	0.003	3.5	0.004	8.2	0.006	8.8
Total	*0.161*[c]	100	0.096	100	0.086	100	0.049	100	0.068	100
Leaf (Phenyl-label)										
Acetone surface wash	16.0	93.7	9.9	86	6.8	81	4.0	61	2.9	54
Extract (acetone, acetone/water, & acetone/HCl)	1.0	6.1	1.2	11	1.2	14	1.8	28	1.8	34
PES	0.038	0.2	0.38	3.3	0.46	5.4	0.72	11	0.69	13
Total	17.1	100	11.5	100	8.5	100	6.6	100	5.4	100
Leaf (Pyrazole-label)										
Acetone surface wash	16.9	97.5	11.3	92.3	8.2	83	4.9	72	2.9	57
Extract (acetone, acetone/water, & acetone/HCl)	0.40	2.3	0.77	6.4	1.4	14	1.5	22	1.8	35
PES	0.021	0.1	0.17	1.2	0.23	2.3	0.39	5.8	0.45	8.9
Total	17.3	100	12.3	100	9.9	100	6.8	100	5.1	100

[a] expressed as mg pyflubumide equivalents/kg

[b] washed sample was not extracted because the combustion analysis indicated that the radioactive residues in washed sample was < 0.01 mg eq/kg.

[c] sum of radioactivity in washes and that in washed fruit by combustion.

Identification of radioactive metabolites was attempted for washes and extracts by using HPLC and TLC. The distribution and identification of radioactive residues in apple fruit and leaf treated with radio-labelled pyflubumide at a nominal rate of 400 g ai/ha are given in Table 4. Total amounts in washes and extracts are shown in the table.

Regardless of the position of ^{14}C, the predominant radioactive residue was the parent pyflubumide. In fruit, it accounted for 88–92% TRR (0.14–0.17 mg eq/kg) on DAT 0, 50–54% TRR (0.048–0.049 mg eq/kg on DAT 7 and 19–28% TRR (0.013–0.016 mg eq/kg) on DAT 51.In leaf, it accounted for 95–96% TRR (16 mg eq/kg) on DAT 0, 56% TRR (6.4–6.9 mg eq/kg) on DAT 7 and 17–22 % TRR (0.89–1.2 mg eq/kg) on DAT 51. On DAT 51, the parent was still the most abundant identified residue.

After the treatment, P-NH was the only identified metabolite. In fruit, P-NH accounted for only 1.2–2.7% TRR (< 0.01 mg eq/kg) on DAT 0 and increased to 15–16% TRR (0.014 mg eq/kg) on DAT 7 and peaked at 15–17% (0.013–0.018 mg eq/kg) on DAT 14. In leaf, it accounted for 1.9–2.7% TRR (0.33–0.46 mg eq/kg) on DAT 0, 11–14% TRR (1.3–1.6 mg eq/kg) on DAT 7 and peaking at 14–15% TRR (1.3–1.4 mg eq/kg) on DAT 14 and after which gradually decreased. Many metabolites/degradates co-chromatographed with P-NH in HPLC. Two-dimensional TLC of the P-NH fraction from the leaf sample after the treatment with phenyl-label showed lower concentration of P-NH.

The remainder of the radioactive residues consisted of multiple (up to 23 in surface washes), minor peaks in HPLC and each accounted for < 0.01 mg eq/kg and <10% of TRR.

Each of the treatments with 1M and 6M HCl and 1M KOH of the leaf PES from DAT 28 and 51 samples at 50 °C for 4 hours released only < 1% TRR. The treatment with 24% KOH at 25 °C for 16.5 hours released 3.9 to 5.0% and 1.5–2.4% TRR for the phenyl-label treatment and pyrazole-label treatment respectively. Most radioactivity in PES still remained unextracted.

The treatment with beta-glucosidase on the surface wash and extracts from 51 DAT leaf sample did not release radioactive compounds.

Table 4 Distribution and identification of components in washes and extracts of fruit and leaf samples from apple trees treated with radioactive pyflubumide at a nominal rate of 400 g ai/ha.

Component	DAT 0		DAT 7		DAT 14		DAT 28		DAT 51	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
	Fruit, Phenyl-label									
Pyflubumide	0.17	92	0.049	54	0.045	42	0.018	27	0.016	28
P-NH[a]	0.005	2.7	0.014	16	0.018	17	0.012	18	0.009	16
Unidentified (total)	0.01	5.3	0.023	26	0.038	35	0.029	43	0.025	43
	Fruit, Pyrazol-label									
Pyflubumide	0.14	88	0.048	50	0.031	36	0.015	31	0.013	19
P-NH[a]	0.002	1.2	0.014	15	0.013	15	0.008	16	0.01	15
Unidentified (total)	0.014	8.7	0.032	33	0.039	45	0.022	45	0.039	57
	Leaf, Phenyl-label									
Pyflubumide	16.4	96	6.4	56	3.2	38	2.0	30	1.2	22
P-NH[a]	0.332	1.9	1.6	14	1.3	15	0.88	13	0.66	12
Unidentified (total)	0.364	2.1	3.1	27	3.6	42	3.0	45	2.8	53
	Leaf, Pyrazole-label									
Pyflubumide	16.4	95	6.9	56	4.0	41	1.7	26	0.89	17
P-NH[a]	0.46	2.7	1.3	11	1.4	14	0.89	13	0.63	12
Unidentified (total)	0.45	2.6	3.9	32	4.2	43	3.8	56	3.1	61

[a] including multiple unidentified metabolites. Each unidentified metabolite 0.21 mg eq/kg (1.8% TRR)

When treated once with radio-labelled pyflubumide each at a nominal rate of 400 g ai/ha, the distribution of radioactivity in fractions was similar for the two radiolabels (phenyl and pyrazole). The majority of radioactive residues were recovered in acetone surface washes of fruit and leaf fractions and the percentage of TRR and concentration decreased significantly over the course of 51 days. The major radioactive residue in the surface washes and extracts in fruit and leaf samples was the parent pyflubumide which decreased in the course of 51 days. Only one metabolite, P-NH was found at > 10% TRR and > 0.01 mg eq/kg on DAT 7 and later, with a peak on DAT 14 in both fruit and leaf samples. Other radioactive residues than the parent and P-NH were found in the surface wash or extracts of either of plant fractions < 10% TRR and < 0.01 mg eq/kg. A small proportion of radioactive residues remained unextracted but over time the ratio of unextracted residue increased

over time (up to about 13% TRR on DAT 51). Minor metabolites were detected but none of which was above 10% TRR or < 0.01 mg eq/kg.

Eggplant (Masaki T., 2012, R-32003)

The metabolism of pyflubumide in eggplant (variety Senryou 2) grown in pots (one for the control, and two for treatment) in the greenhouse equipped with a quartz glass ceiling to allow a full range of irradiation of sun light in Japan. No information was available on the soil type.

Both the phenyl- and pyrazole-labelled pyflubumide were formulated as a 20% SC formulation and individually applied during fruit growth stage over the whole plants at a target rate of 600 g ai/ha (actual rate: 490 g ai/ha and 550 g ai/ha, respectively)(concentration, 20 g ai/hL), while the soil was covered with paper.

Duplicate fruits and 3-4 leaves were sampled at 0, 7 and 14 days after treatment. Root samples were taken only at the last sampling and dried. Immediately after sampling, samples were washed with acetone and extracted twice with acetone and a mixture of acetone and water (1/1, v/v). Equal volumes of acetone extract and acetone/distilled water (1/1) extract were combined and then partitioned into ethyl-acetate and acetonitrile after addition of ammonium sulfate. All organic phases were combined to constitute the extracted fraction and concentrated for chromatographic analysis. Washes and extracts of each sample were stored in a freezer during this study. PES and dry root were combusted to determine unextracted radioactivity. To improve combustion efficacy, an aliquot of dried PES and root were mixed with cellulose powder prior to combustion and subsequent measurement by LSC.

Metabolite characterization and identification for eggplant leaf, fruit and roots was accomplished by radiochemical and chromatographic methods (TLC and HPLC-RI). For chromatographic identification and quantification of radioactive residues, aliquots of concentrated extract were applied to silica-gel TLC chromatography. Radio-luminograms were superimposed to chromatograms of the reference substances visualized by UV irradiation. For identification of radioactive components, radioactivity in washes and extracts from leaves was chromatographed on HPLC followed by measurement of radioactivity. Radio-chromatogram was obtained by plotting determined radioactivity versus collected time. Identities of radioactive components were confirmed by comparison with UV absorbance of co-chromatographed reference substances.

The TRR in the fruits and leaves are shown in Table 5. The TRR showed similar tendencies between the two radiolabelled pyflubumide. In the fruits, TRR on DAT 0 of radioactive pyflubumide at a nominal rate of 600 g ai/ha were 0.76–1.4 mg eq/kg and on DAT 14 1.0 mg eq/kg. The TRR in the leaves were much higher at 55–75 mg eq/kg on DAT 0 and showed decrease to 20–44 mg eq/kg on DAT 14. Since only a small amount of radioactive residues were detected in root (< 0.03 mg eq/kg) on DAT 14, translocation of radioactivity from the sprayed plant parts to roots seems insignificant.

Table 5 Total radioactive residues in the fruit and leaf samples from eggplant treated with radioactive pyflubumide at a nominal rate of 600 g ai/ha.

Label	TRR (mg pyflubumide equivalents/kg)		
	DAT 0	DAT 7	DAT 14
	Fruit		
Phenyl-label	1.41	0.66	1.00
Pyrazole-label	0.76	0.88	1.02
	Leaf		
Phenyl-label	74.5	47.7	43.7
Pyrazole-label	55.4	30.6	19.8
	Roots		
Phenyl-label	-	-	< 0.01

Label	TRR (mg pyflubumide equivalents/kg)		
	DAT 0	DAT 7	DAT 14
Pyrazole-label	-	-	0.03

The distribution of radioactivity in the acetone surface wash, acetone/water extract and in the PES of each sample is shown in Table 6.

The distribution of radioactivity in fruit fractions showed similarity between the two types of radiolabeled pyflubumide. More than 95% of the TRR was recovered in acetone washes and extracts. From DAT 0 through DAT 14, the largest portion of radioactivity was recovered from the washes accounting for 93–99% TRR and 0.64–1.4 mg eq/kg in fruits and 86–96% TRR and 18–69 mg eq/kg in leaves. Combined radioactive residues extracted with acetone and acetone/water from the washed fruits accounted for 0.96–2.5% TRR and from the washed leaves, 4.3–12% TRR, with those from the phenyl-label treatment showing higher TRR and concentrations. Although no significant radioactivity (≤ 5.2% TRR) was found in PES, radioactive residues continues to increase over time from virtually zero on DAT 0. The radioactive residues in PES of the leaf sample from the phenyl-label reached 5.2% TRR and 2.23 mg eq/kg, but no characterization of unextracted radioactivity was attempted.

Table 6 Radioactive residues in fractions of the fruit and leaf samples from eggplant treated with radioactive pyflubumide at a nominal rate of 600 g ai/ha

Fraction	DAT 0		DAT 7		DAT 14	
	mg/kg [a]	% TRR	mg/kg [a]	% TRR	mg/kg [a]	% TRR
	Fruit (Phenyl-label)					
Acetone surface wash	1.37	97.7	0.64	97	0.94	93
Acetone extract	0.02	1.6	0.01	1.91	0.02	1.60
Acetone/water extract	< 0.01	0.51	< 0.01	0.39	< 0.01	0.09
Wash + extracts	1.40	99.8	0.66	99.2	0.96	95.0
PES	< 0.01	0.19	< 0.01	0.81	0.04	4.98
Total	1.41	100	0.66	100	1.0	100
	Fruit (Pyrazole-label)					
Acetone surface wash	0.72	99	0.85	98	0.96	95
Acetone extract	< 0.01	0.82	0.01	1.4	0.02	1.8
Acetone/water extract	< 0.01	0.14	< 0.01	0.11	< 0.01	0.7
Wash + extracts	0.73	100	0.87	99	0.99	98
PES	ND	ND	< 0.01	0.98	0.03	2.5
Total	0.73	100	0.88	100	1.02	100
	Leaf (Phenyl-label)					
Acetone surface wash	69.2	93.1	40.8	85.7	37.4	85.6
Acetone extract	5.0	6.6	5.3	11	3.8	8.6
Acetone/water extract	0.22	0.29	0.47	0.96	0.27	0.64
Wash + extracts	74.5	100	46.5	97.5	41.5	94.8
PES	0.02	0.02	1.2	2.5	2.2	5.2
Total	74.5	100	47.7	100	43.7	100
	Leaf (Pyrazole-label)					
Acetone surface wash	52.9	95.6	28.6	94.1	18.34	92.5
Acetone extract	2.3	4.2	1.7	5.2	0.97	4.9
Acetone/water extract	0.11	0.19	0.16	0.49	0.11	0.55
Wash + extracts	55.3	100	30.5	99.8	19.42	98.0
PES	0.02	0.02	0.07	0.23	0.40	2.0
Total	55.4	100	30.6	100	19.82	100

[a] expressed as mg pyflubumide equivalents/kg

ND not detected

Identification of radioactive metabolites was attempted by using HPLC and TLC co-chromatography on washes and extracts. The distribution and identification of residues in eggplant fruit and leaf samples after treatment with radiolabelled pyflubumide at a nominal rate of 600 g ai/ha are given in Table 7.

The predominant radioactive residue in the extracts was the parent pyflubumide, regardless of the position of radiolabel throughout the study period, accounting for of 90–98% (0.63–1.4 mg eq/kg) in fruits; and 90–99% TRR (19–74 mg eq/kg) in leaves. Pyflubumide decreased in leaves although the percentage of TRR did not change significantly. Radioactive residues increased over time to the maximum of 5.2% TRR in fruits and leaves.

From the phenyl-label treatment, P-NH, P-NH-RfOH were detected from fruits (washes and extracts) at < 1.2% TRR and < 0.01 mg eq/kg. P-aniline isobutyryl was detected only on DAT 14 in the fruit (washes and extracts) at 1.0% TRR (0.01 mg eq/kg). In leaves, P-NH, P-NH-RfOH and P-aniline-isobutyryl accounted for at the maximum 1.3% TRR (up to 0.59 mg eq/kg).

From the pyrazole-label treatment, P-NH and P-acid were detected from fruits at < 1% TRR and < 0.01 mg eq/kg. Radioactive residues remained in PES were either not detected on DAT 0 and 7 and < 2.5% TRR and 0.03 mg eq/kg on DAT 14. In leaves, P-NH was detected at < 1% TRR (0.15–0.24 mg eq/kg) throughout the study, and P-acid up to 0.27 % TRR and 0.02–0.06 mg eq/kg. P-NH-RfOH and P-NH-5-CH_2OH were detected only on DAT 14 at < 0.01 mg eq/kg (0.02% TRR),

No other peaks were further identified.

Table 7 Distribution and identification of components in fruit and leaf samples from eggplant treated with radioactive pyflubumide at a nominal rate of 600 g ai/ha.

Component	DAT 0		DAT 7		DAT 14	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
	Fruit (Phenyl-label)					
Pyflubumide	1.38	98.2	0.63	96	0.90	90
P-NH	< 0.01	0.40	< 0.01	0.83	0.01	1.2
P-NH-RfOH	ND		ND		< 0.01	0.02
P-aniline-isobutyryl	< 0.01	0.63	< 0.01	0.87	0.01	1.0
Others	< 0.01	0.05	< 0.01	1.2	0.03	3.1
TLC origin	ND		ND		< 0.01	0.01
Unidentified extract	< 0.01	0.51	< 0.01	0.39	< 0.01	0.09
Aqueous residue	ND		ND		ND	
PES	< 0.01	0.19	< 0.01	0.81	0.04	5.0
Total	1.41	100	0.66	100	1.00	100
	Fruit (Pyrazole-label)					
Pyflubumide	0.72	98	0.83	95.4	0.98	96
P-NH	< 0.01	0.37	< 0.01	0.52	< 0.01	0.45
P-acid	< 0.01	0.32	< 0.01	0.09	< 0.01	0.02
Others	ND		0.02	1.51	< 0.01	0.58
TLC origin	ND		ND		< 0.01	0.17
Unidentified extract	< 0.01	0.96	0.01	1.55	-	-
Aqueous residue	ND		ND		< 0.01	0.32
PES	ND		< 0.01	0.98	0.03	2.52
Total	0.73	100	0.88	100	1.02	100
	Leaf (Phenyl-label)					

Component	DAT 0		DAT 7		DAT 14	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
Pyflubumide	73.7	99.0	44.2	92.6	39.3	89.9
P-NH	0.34	0.46	0.49	1.02	0.59	1.30
P-NH-RfOH	ND		0.03	0.07	0.06	0.13
P-aniline-isobutyryl	0.42	0.57	0.39	0.82	0.20	0.44
Others	ND		1.4	2.7	1.2	2.7
TLC origin	ND		0.15	0.33	0.17	0.39
Aqueous residue	ND		ND		ND	
PES	0.02	0.02	1.2	2.5	2.2	5.2
Total	74.5	100	47.7	100	43.7	100.0
	Leaf (Pyrazole-label)					
Pyflubumide	55.0	99.3	27.5	91.3	19.0	95.8
P-NH	0.21	0.39	0.24	0.80	0.15	0.73
P-NH-RfOH	ND		ND		< 0.01	0.02
P-NH-5-CH_2OH	ND		ND		< 0.01	0.02
P-acid	0.06	0.11	0.06	0.27	0.02	0.07
Others	0.09	0.17	2.66	7.29	0.21	1.08
TLC origin	ND		0.03	0.10	0.04	0.21
Aqueous residue	0.01	0.02	0.01	0.04	0.02	0.11
PES	0.02	0.02	0.07	0.23	0.40	2.01
Total	55.4	100	30.6	100	19.8	100

ND not detected

When treated with radiolabelled pyflubumide once at a nominal rate of 600 g ai/ha, radioactive residues in fruits and leaves showed similar tendency and distribution. Most of TRR was recovered in washes and extracts. In the study, the predominant radioactive residue in the washes and extracts of fruits and leaves was the parent, pyflubumide (> 90% TRR). Minor metabolites identified were P-NH, P-NH-RfOH, P-NH-acid and P-aniline-isobutyryl, all of which accounted for less than 1.2% TRR in fruits and leaves.

Spinach (Masaki T., 2012, R-32002)

The metabolism of pyflubumide in spinach (variety Sunlight) grown in pots (diameter of 30 cm) in a greenhouse equipped with a quartz ceiling to allow irradiation by sunlight in Japan. There were three pots for a control and treatments with phenyl- or pyrazole-labelled pyflubumide.

Both the phenyl- and pyrazole-labelled pyflubumide were formulated as an SC formulation and applied once over the whole plant at a target rate of 600 g ai/ha (actual rate: 550 g ai/ha and 570 g ai/ha respectively)(concentration, 20 g ai/hL) with the soil covered with paper. Duplicated plants including red roots were harvested at 0, 1, 7, 14 and 21 days after treatment. New leaves appearing after the application and roots were sampled only at final sampling point and dried for combustion.

All samples were extracted immediately after they were harvested at the laboratory on the day of collection. Leaves were washed with chloroform and then homogenized and extracted three times with 3-fold volumes of acetone and then with acetone/distilled water (1/1). Acetone extracts and acetone/distilled water (1/1) extract were combined and then partitioned into ethyl-acetate and acetonitrile in the presence of ammonium sulfate. For the DAT 0 and DAT 1 samples, acetone/water (1/1) extracts were directly subjected to extraction with hexane/ethyl-acetate (1/1) without addition of ammonium sulfate, and subsequently combined with equivalent amounts of acetone extracts. All organic phases were combined to constitute the “extracts” fraction and concentrated for chromatographic analysis. Washes and extracts of each sample were stored in a freezer during the

course of this study. PES and dry root samples were combusted for the determination of radioactivity by LSC. To improve combustion efficacy, an aliquot of dried PES and root were mixed with cellulose powder prior to combustion.

Metabolite characterization and identification for spinach leaves was conducted by radiochemical and chromatographic techniques (TLC and HPLC-RI). For chromatographic identification and quantification of radioactive residues, aliquots of concentrated extract were applied to silica-gel TLC chromatography. Radio-luminograms were superimposed to chromatograms of the reference substances visualized by UV irradiation. For identification of radioactive components, radioactivity washed and extracted from leaves was chromatographed by HPLC followed by measurement of radioactivity of fractionated eluate. Identities of radioactive components were confirmed by comparison with UV absorbance of co-chromatographed. reference substances.

The TRR in the spinach fractions are shown in Table 8. The TRR showed similar tendencies between the two radiolabeled pyflubumide and decreased from 12–13 mg eq/kg at DAT 0 to about 5–7 mg eq/kg at DAT 14–21. The TRR in roots and new leaves were < 0.01 or 0.03 mg eq/kg on Day 21, indicating that there is no significant translocation to roots or new leaves from the treated plant parts after the treatment.

Table 8 Total radioactive residues in the samples from spinach treated with radioactive pyflubumide at a nominal rate of 600 g ai/ha.

Label	TRR (mg pyflubumide equivalents/kg)				
	DAT 0	DAT 1	DAT 7	DAT 14	DAT 21
	Leaf				
Phenyl-label	12.7	-	-	4.7	7.1
Pyrazole-label	12.4	13.8	8.2	5.8	5.9
	Root				
Phenyl-label	-	-	-	-	< 0.01
Pyrazole-label	-	-	-	-	0.01
	New leaves				
Phenyl-label	-	-	-	-	< 0.01
Pyrazole-label	-	-	-	-	0.03

-: not sampled

The distribution of radioactivity in the surface washes, acetone and acetone/water extracts and in the PES of each sample is shown in Table 9 and Table 6. The distribution of radioactive residues in the fractions was similar between the two radiolabelled pyflubumide.

Throughout the study period, almost all of the radioactivity on or in leaves were either in the washes or in extracts (close to 100%). The most of these radioactive residues were recovered from washes which accounted for 80–92% TRR. The acetone and acetone/water extracts accounted for 7.8–18% TRR and 0.3–1.5% TRR, respectively. The PES only insignificant amounts of radioactive residues (< 0.4% TRR and < 0.02 mg eq/kg).

Table 9 Distribution of radioactivity in spinach fractions following application of phenyl- or pyrazole-labelled pyflubumide

Fraction	DAT 0		DAT 1		DAT 7		DAT 14		Day 21	
	mg/kg [a]	% TRR	mg/kg [a]	% TRR	mg/kg [a]	% TRR	mg/kg [a]	% TRR	mg/kg [a]	% TRR
	Phenyl-label									
Chloroform surface wash	11.7	91.9	-	-	-	-	4.1	86	6.3	87
Acetone extract	0.97	7.8	-	-	-	-	0.59	13	0.78	12

Fraction	DAT 0		DAT 1		DAT 7		DAT 14		Day 21	
	mg/kg [a]	% TRR	mg/kg [a]	% TRR	mg/kg [a]	% TRR	mg/kg [a]	% TRR	mg/kg [a]	% TRR
Acetone/water extract	0.03	0.3	-	-	-	-	0.05	1.0	0.07	1.0
Wash + extracts	12. 7	100	-	-	-	-	4.7	99.7	7.1	100
PES	< 0.01	< 0.1	-	-	-	-	0.01	0.3	0.02	0.2
Total	12. 7	100	-	-	-	-	4.7	100	7.1	100
	Pyrazole-label									
Chloroform surface wash	10.7	87.1	11.6	83.8	7.0	85	4.7	80	5.0	83
Acetone extract	1.6	12	2.1	16	1.1	14	1.0	18	0.87	15
Acetone/water extract	0.06	0.5	0.08	0.6	0.06	0.8	0.09	1.5	0.08	1.4
Wash + extracts	12.4	100	13.8	100	8.2	99.8	5.8	99.6	5.9	100
PES	< 0.01	< 0.1	< 0.01	< 0.1	0.02	0.2	0.02	0.4	0.02	0.4
Total	12.4	100	13.75	100	8.2	100	5.8	100	5.9	100

[a] expressed in mg pyflubumide equivalents/kg

- not sampled

The identification and characterization of radioactive residues in washes and extracts of spinach samples after treatment with radiolabelled pyflubumide at a nominal rate of 600 g ai/ha are given in Table 10. Since only minor amount of radioactivity (< 0.1% TRR, < 0.02 mg eq/kg) was detected in PES, no characterization of radioactivity in PES was conducted.

In the washes and extracts, the predominant radioactive component was the parent compound, pyflubumide accounting for 84–100% TRR. Minor radioactive metabolites were detected: P-NH (up to 3.2% TRR, 0.19 mg eq/kg) and P-acid (up to 0.8% TRR, 0.05 mg eq/kg). No identified metabolites were above 3.3% TRR. Small amounts of unknown metabolites (up to 5.0% TRR, 0.28 mg eq/kg) and up to 6.3% TRR remained at the TLC origin.

Unknown-1 (U-1) was detected in the extracts from the treatments with either of radio-labelled pyflubumide. As result of mass spectrometry, the molecular weight of U-1 was determined to have a m/z ratio of 536, which is equivalent to the one of pyflubumide and considered to be a position-isomer of the parent. Since the amount of purified U-1 was insignificant for further analysis, such as by NMR, no further analysis was made.

Another minor radioactivity of unknown-2 (U-2) (4.1% TRR, 0.25 mg eq/kg) and unknown 3 (U-3) (0.4% TRR, 0.05 mg eq/kg) was also detected in extracts but, since they were suspected to be artifacts as their detection was incidental and not further characterization was conducted.

Table 10 Identification and characterization of radioactive residues in spinach following the application of phenyl- or pyrazole-labelled pyflubumide

Component	DAT 0		DAT 1		DAT 7		DAT 14		DAT 21	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
	Phenyl-label									
Pyflubumide	12. 7	100	-		-		4.4	93	6.4	91
P-NH	ND		ND		ND		0.03	0.7	0.16	2.2
TLC origin	ND		ND		ND		0.11	2.5	0.19	2.9
Unknown 1	ND		ND		ND		0.15	3.2	0.31	4.1
Aqueous unextracted	< 0.01	< 0.1	ND		ND		< 0.01	< 0.01	< 0.01	< 0.1

Component	DAT 0		DAT 1		DAT 7		DAT 14		DAT 21	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
Total	12.7	100	ND		ND		4.7	100	7.1	99.7
	Pyrazole-label									
Pyflubumide	12.3	99.3	13.7	99.8	7.8	95	4.9	83	5.0	84
P-NH	0.03	0.2	ND		ND		0.12	2.1	0.19	3.2
P-acid	ND		ND		ND		ND	ND	0.05	0.8
TLC origin	ND		ND		0.13	1.6	0.25	4.4	0.36	6.3
Unknown 1	ND		ND		0.25	3.0	0.24	4.1	0.28	5.0
Others	0.05	0.4	ND		ND	ND	0.25	4.1	< 0.01	0.1
Aqueous unextracted	< 0.01	< 0.1	0.01	0.1	0.02	0.3	0.09	1.6	0.04	0.7
Total	12.4	100	13.8	99.9	8.2	99.8	5.8	99.6	5.9	99.6

- not sampled

The polar fraction (radioactivity remaining at the TLC-origin after TLC) was subjected to chemical characterization through enzymatic and acidic hydrolysis. Radioactive components that appeared after enzymatic reaction with beta-glucosidase are summarized in Table 11.

The polar fraction from the samples collected DAT 14 and 28 from the pyrazole-label treatment showed that radioactivity at TLC origin partially diminished and a small amount of P-acid (1.4% TRR) was liberated after the enzymatic reaction, suggesting that part of the radioactivity at TLC origin was possibly a glucose conjugate of P-acid. In addition, a number of radioactive compounds (in total < 1.1% TRR) which could not be assigned to the reference standards by TLC were also liberated.

The polar fraction from the phenyl-label treatment did not release significant radioactivity by the enzyme reaction. With acidic hydrolysis, the radioactivity of TLC origin decreased, but only insignificant radioactivity was detected due to further degradation of the hydrolysate.

Table 11 Enzymatic hydrolysis of the polar radioactive fraction remaining at the TLC origin

Compound or fraction	DAT 14				DAT 21			
	Pre		Post		Pre		Post	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
	Phenyl-label							
Others			0.02	0.5			0.05	0.8
TLC origin	0.12	2.5	0.11	2.5	0.19	2.9	0.09	1.5
Total	0.12	2.5	0.14	3.0	0.19	2.9	0.14	2.3
	Pyrazole-label							
P-acid			0.08	1.4	0.05	0.8	0.08	1.3
Others			0.06	1.1			0.06	1.1
TLC origin	0.25	4.4	0.15	2.5	0.36	6.3	0.17	2.9
Total	0.25	4.4	0.29	5.1	0.41	7.1	0.31	5.2

Summary of Plant Metabolism

Metabolism of pyflubumide in plants after one foliar application was studied on apple, eggplant and spinach plants using phenyl- and pyrazole-labelled pyflubumide formulated as SC formulations.

The studies on eggplant and spinach indicated that there was no significant translocation from the sprayed parts of plants to roots or new leaves. Most radioactive residues were on the surface of

fruits or leaves. Additional radioactive residues were extracted by acetone and acetone/water (1:1). PES contained only very minor proportion of the radioactivity.

The predominant extracted radioactive residue was the parent, pyflubumide. Some or minor amounts of metabolites, P-NH (apple, eggplant and spinach), P-NH-RfOH (eggplant), P-aniline-isobutyryl (eggplant), P-NH-5-CH_2OH (eggplant) and P-acid (eggplant and spinach) were identified. However, the only metabolite that was present >10% TRR and > 0.01 mg/kg was P-NH (only in apple). All metabolites, except P-NH-5-CH_2OH were also detected in rat metabolism studies.

Taking into consideration the plant metabolism studies, identified metabolites in these studies and short PHI specified in GAP in Japan, the majority of pyflubumide remains unchanged, or produces P-NH by elimination of the isobutyryl group from the amide nitrogen.

ANIMAL METABOLISM

No animal metabolism studies were submitted to the Meeting. Supervised residue trial results submitted to the Meeting were on apple and tea. Tea commodities are not listed among animal feed stuffs. While apple pomace can be fed to livestock, the calculated dietary burden from apple pomace was < 1 mg/kg.

Metabolism studies on laboratory animals including rats were reviewed in the framework of the toxicological evaluation by the current JMPR and the relevant information is summarized below.

Rat

Rat metabolism studies were conducted using a single oral dose of phenyl- (1 mg/kg bw) or pyrazole-labelled (1 and 100 mg/kg bw) pyflubumide to male and female rats. Following oral administration to rats of ^{14}C-radiolabelled pyflubumide as a single dose of 1 mg/kg bw, the compound was rapidly absorbed (T_{max} 6 h) but only partially. Based on urinary (< 6%) and biliary (ca 43%) excretion, cage wash, tissue and carcass residues after 72 hours, absorption accounted for ca 52% of the applied dose. Absorption of a single dose of 100 mg/kg bw was expected to be only marginally lower. The absorbed portion was widely distributed throughout the body, with highest concentrations found in liver and kidneys, adrenals, bone marrow and fat. Elimination was nearly complete at the low and high dose level after 7 days with faeces being the main route of elimination, accounting for 90% or more. In nursing rats, excretion of pyflubumide and of some of its metabolites via the milk was demonstrated. The milk:plasma radioactivity ratio was approximately 10:1, and the area under the concentration–time curve (AUC) was up to 7.5 times higher in milk than in plasma.

Extensive metabolism of pyflubumide was observed, at least of the systemically available portion. The main metabolic pathways comprised deacylation of the nitrogen atom, followed by hydroxylation and demethylation, whereas cleavage of the molecular backbone of pyflubumide was very limited. Eight or nine metabolites were identified in urine, feces, plasma or milk, but each displayed a different mix. In bile there were 12 metabolites. Main metabolites (exceeding 10% of administered dose in either excreta or plasma in ADME studies) were P-NH, P-NH-1-H-RfOH and P-NH-1-H-3′-(3-OH)-RfOH. The unchanged parent compound was mainly detected in the gastrointestinal tract and faeces, representing the non-absorbed part, but to a small extent also in milk.

The impact of sex, dose, or position of radiolabel on metabolism was low.

All metabolites identified in the plant metabolism studies, except P-NH-5-CH2OH, were also reported in rat metabolism.

ENVIRONMENTAL FATE

The Meeting received information on hydrolytic degradation and photodegradation in aquatic system and aerobic degradation in soil. Since pyflubumide is used as foliar spray, the submitted data are in line with the requirements of OECD and described in the FAO Manual.

Hydrolytic degradation (Masaki T., 2012; E-32001)

Eight mL of phosphate buffer solution and 0.8 µg eq. of each labelled test substance dissolved in 40 µL acetonitrile were pipetted into sterilized brown glass vial (20 mL) with screw cap to the target concentration of 0.01 mg/L. Final concentration of acetonitrile in the test solution was below 1.0%.

Tier 1 testing was performed at 50 ± 0.5 °C for pH 4.0, 7.0 and 9.0 for 5 days. Since degradation beyond 10% of applied radioactivity was observed, a Tier 2 test was conducted. In Tier 2 testing, samples were withdrawn from each solution from day 1 to day 30 except that for higher temperatures at pH 9 samples were withdrawn up to 2 days (40 °C) or 0.5 day (50 °C).

The test solution obtained from the test vessel was extracted twice with 4 mL ethyl acetate. Prior to extraction, test solution at pH 7 and pH 9 was acidified by 0.1 N HCl. The volume of extract was adjusted to 10 mL with ethyl acetate to be subjected to determination of total radioactivity. Aliquots of concentrated extract and reference standard were applied to TLC plates for two-dimensional chromatography.

For chromatographic identification of radioactive components, selected extracts were subjected to HPLC analysis after concentration and reconstitution with acetonitrile/distilled water (1/1). Identities of radioactive components were confirmed by comparison with UV absorbance of co-injected reference substances.

Degradation rate constants were determined by the least-square regression method based on natural logarithm (Ln) of concentration as dependent parameter and time duration as independent parameter. Degradation rate constant as a function of temperatures was calculated based on the Arrhenius equation.

In Tier I test, hydrolysis of the test substances at 50 °C for 5 days were greater than 10% of applied radioactivity at all the tested pH values leading to Tier 2.

The results of identification and quantification of radioactivity in the buffer solutions incubated at 25, 40 and 50 °C are shown in the table below. Overall radioactive recovery ranged between 97-105% showing no significant loss of radioactivity during incubation. Most of radioactivity was extracted, which accounted for more than 96% of applied radioactivity. Radioactivity remaining in aqueous phase after extraction was less than 4.5% of applied radioactivity. Chromatographic analysis of extracted radioactivity indicated the pyflubumide was significantly hydrolysed under all the tested pH and temperatures.

Hydrolysis of test substances were more significant at pH 9 than at pH 4 or pH 7. Regardless of pH, pyflubumide was mainly hydrolysed to three radioactive components: P-NH, P-aniline-isobutyryl and P-acid, which accounted for, at the maximum, 95%, 34% and 26%, respectively. These major hydrolysates occurred above 10% of applied radioactivity throughout all tested conditions. P-NH, a major hydrolysate reached the maximum concentration pH 9, while P-aniline-isobutyryl and P-acid appeared to occur at higher concentrations under pH 7 and pH 4.

Table 12 Hydrolytic degradation of radio-labelled pyflubumide at pH 4, 7 and 9 at different temperatures

Fraction Component	**% of Applied radioactivity (At specified days after treatment)**							
	pH 4 at 25 °C (phenyl-label)							
	0	1	3	7	14	21	30	
Extract	100	100	102	101	100	101	99	
Pyflubumide	99	98	94	88	72	63	49	
P-NH	0.8	1.6	4.5	8.6	15	21	25	
P-aniline-isobutyryl	0.4	0.8	2.7	4.7	13	17	25	
Aqueous phase	2.5	1.1	1.5	0.3	0.8	0.3	0.6	

Fraction Component	% of Applied radioactivity (At specified days after treatment)							
Total	103	102	103	102	101	102	100	
	pH 4 at 25 °C (pyrazole-label)							
	0	1	3	7	14	21	30	
Extract	101	100	102	100	100	99	99	
Pyflubumide	100	98	96	89	82	70	59	
P-NH	0.9	1.4	3.9	7.7	12	18	25	
P-acid	0.2	0.3	1.1	3.0	5.4	11	15	
Aqueous phase	0.9	0.9	1.3	1.3	1.8	3.1	3.5	
Total	102	101	103	101	101	102	103	
	pH 7 at 25 °C (phenyl-label)							
	0	1	3	7	14	21	30	
Extract	100	100	100	100	100	101	101	
Pyflubumide	99	96	91	81	67	53	45	
P-NH	0.9	2.5	6.3	13	22	28	33	
P-aniline-isobutyryl	0.3	1.1	2.1	6.2	11	20	23	
Aqueous phase	1.8	0.6	0.8	0.4	0.6	0.5	0.5	
Total	102	100	100	101	101	102	102	
	pH 7 at 25 °C (pyrazole-label)							
	0	1	3	7	14	21	30	
Extract	101	100	100	100	98	98	97	
Pyflubumide	100	98	92	86	68	60	51	
P-NH	1.1	2.1	6.5	11	22	25	33	
P-acid	0.2	0.6	1.2	3.0	8.3	13	13	
Aqueous phase	0.9	0.9	1.6	1.0	2.6	3.3	3.1	
Total	102	101	101	101	101	101	101	
	pH 9 at 25 °C (phenyl-label)							
	0	0.125	0.25	0.5	1	3	10	30
Extract	101	102	101	99	101	100	100	101
Pyflubumide	99	95	90	82	72	58	35	3.5
P-NH	0.8	5.5	10	15	27	36	54	8 1
P-aniline-isobutyryl	0.5	0.9	1.2	1.9	2.6	4.6	10	17
Aqueous phase	1.4	1.8	0.9	1.7	0.7	0.4	0.1	0.6
Total	102	103	102	101	101	100	100	102
	pH 9 at 25 °C (pyrazole-label)							
	0	0.125	0.25	0.5	1	3	10	30
Extract	101	102	101	100	100	100	99	98
Pyflubumide	100	96	90	85	74	54	26	7.8
P-NH	1.0	5.8	9.6	13	24	43	66	79
P-acid	0.2	0.6	0.7	1.2	1.6	2.5	6.6	12
Aqueous phase	0.7	1.4	1.0	1.9	1.4	1.5	1.9	2.7
Total	102	103.4	102	101	101	101	101	101
	pH 4 at 40 °C (phenyl-label)							
	0	1	3	5	7	11	14	30
Extract	100	102	101	102	100	102	102	100
Pyflubumide	99	93	78	74	62	49	40	18

Fraction Component	% of Applied radioactivity (At specified days after treatment)							
P-NH	0.8	6.0	13	16	22	31	36	47
P-aniline-isobutyryl	0.4	3.0	10	12	16	23	25	34
Aqueous phase	0.7	0.3	0.5	N.D.	0.8	0.1	0.5	0.5
Total	101	102	102	102	101	102	102	100
	pH 4 at 40 °C (pyrazole-label)							
	0	1	3	5	7	11	14	30
Extract	101	100	99	100	98	96	96	100
Pyflubumide	100	94	84	76	62	56	49	23
P-NH	1.2	4.9	9.6	16	23	25	32	51
P-acid	0.0	2.0	5.6	8.8	13	15	15	26
Aqueous phase	1.5	1.5	1.8	2.9	2.9	4.5	4.4	1.2
Total	103	102	101	103	101	101	100	101
	pH 7 at 40 °C (phenyl-label)							
	0	1	3	5	7	11	14	30
Extract	100	100	102	102	100	100	100	99
Pyflubumide	99	91	79	73	67	54	52	15
P-NH	0.7	6.2	15	20	21	31	33	66
P-aniline-isobutyryl	0.3	2.6	7.3	8.8	11	15	15	17
Aqueous phase	1.1	0.8	0.6	0.6	0.8	0.5	0.8	0.2
Total	101	101	102	103	101	100	101	99
	pH 7 at 40 °C (pyrazole-label)							
	0	1	3	5	7	11	14	30
Extract	102	101	100	98	99	98	96	100
Pyflubumide	101	94	84	75	71	64	44	20
P-NH	0.7	5.5	13	17	21	28	39	56
P-acid	0.2	1.3	4.0	6.2	6.7	6.9	13	23
Aqueous phase	1.1	0.9	1.4	2.0	1.7	2.1	3.2	0.6
Total	103	102	102	100	101	101	99	100
	pH 9 at 40 °C (phenyl-label)							
	0	0.042	0.125	0.25	0.5	1	2	
Extract	100	104	104	104	104	104	104	
Pyflubumide	99	92	78	61	50	31	3.9	
P-NH	0.7	10	24	41	50	67	95	
P-aniline-isobutyryl	0.4	1.2	2.0	2.7	3.3	5.7	5.1	
Aqueous phase	N.D.	0.7	0.2	0.5	0.7	0.2	0.2	
Total	100	104	104	104	104	104	104	
	pH 9 at 40 °C (pyrazole-label)							
	0	0.042	0.125	0.25	0.5	1	2	
Extract	102	102	104	103	103	102	102	
Pyflubumide	102	92	76	66	51	29	7.1	
P-NH	0.6	9.1	26	36	50	71	90	
P-acid	0.2	0.5	1.0	1.4	1.8	2.8	4.6	
Aqueous phase	N.D.	0.8	0.2	0.4	0.5	0.7	1.0	
Total	102	102	104	103	103	103	103	

Fraction Component	% of Applied radioactivity (At specified days after treatment)							
	pH 4 at 50 °C (phenyl-label)							
	0	1	2	3	5	10	15	
Extract	101	101	101	101	101	101	99	
Pyflubumide	99	83	68	64	55	48	33	
P-NH	1.5	12.5	23.8	19	35	30	39	
P-aniline-isobutyryl	0.4	5.5	8.9	18	12	22	28	
Aqueous phase	0.3	0.5	N.D.	N.D.	0.4	0.4	1.0	
Total	102	101	101	101	101	101	100	
	pH 4 at 50 °C (pyrazole-label)							
	0	1	2	3	5	10	15	
Extract	100	98	98	98	97	100	98	
Pyflubumide	100	84	72	66	59	52	37	
P-NH	0.6	10	21	25	26	33	41	
P-acid	0.2	4.1	5.8	8.7	12	15	20	
Aqueous phase	1.8	0.9	1.3	1.8	1.1	0.2	0.2	
Total	102	99	100	100	98	100	99	
	pH 7 at 50 °C (phenyl-label)							
	0	1	2	3	5	10	15	
Extract	101	100	101	100	100	100	100	
Pyflubumide	99	80	66	59	57	36	19	
P-NH	1.4	14	25	28	30	44	62	
P-aniline-isobutyryl	0.4	5.9	9.8	13	13	20	19	
Aqueous phase	N.D.	N.D.	N.D.	N.D.	0.2	0.1	1.0	
Total	101	100	101	100	100	100	102	
	pH 7 at 50 °C (pyrazole-label)							
	0	1	2	3	5	10	15	
Extract	101	99	98	98	96	101	99	
Pyflubumide	100	82	68	67	60	50	20	
P-NH	1.3	14	25	25	27	38	56	
P-acid	0.2	2.9	4.7	5.8	8.9	13	23	
Aqueous phase	0.5	0.8	1.0	1.2	0.7	0.3	0.4	
Total	102	100	99	99	97	101	99	
	pH 9 at 50 °C (phenyl-label)							
	0	0.042	0.083	0.125	0.25	0.375	0.5	
Extract	102	102	101.9	103	101	103	102	
Pyflubumide	101	82	69.9	61	48	30	13	
P-NH	0.4	19	30.7	40	52	69	84	
P-aniline-isobutyryl	N.D.	0.9	1.2	1.4	1.8	4.1	4.8	
Aqueous phase	0.6	0.4	0.9	0.4	0.4	N.D.	0.2	
Total	102	102	102.8	103	102	103	102	
	pH 9 at 50 °C (pyrazole-label)							
	0	0.042	0.083	0.125	0.25	0.375	0.5	
Extract	102	101	101.4	101	102	102	101	
Pyflubumide	102	82	69.1	62	51	29	14	

Fraction Component	% of Applied radioactivity (At specified days after treatment)							
P-NH	0.6	19	31.5	38	50	70	84	
P-acid	0.2	0.5	0.7	0.9	1.0	2.9	3.8	
Aqueous phase	*N.D.*	*N.D.*	0.1	0.1	0.9	0.4	0.3	
Total	102	101	101.5	101	103	102	101	

At each pH, half-lives of pyflubumide (both label) at each pH were shorter at higher temperatures. At 25 °C at pH 7, relevant to the environmental condition, half-life of pyflubumide was 27.9 day (mean of both labels) and it was most stable at pH 4 ($t_{1/2}$ = 32.4 days while relatively unstable at pH 9 ($t_{1/2}$ = 6.6 days).

Degradation rate constants of pyflubumide (both labels) were calculated for each pH and temperature and the mean of both labels at pH 7 at 25 °C was 2.5×10^{-2} day^{-1}.

In summary, pyflubumide was significantly hydrolysed at all tested pH and temperatures. Hydrolysis rates were higher at pH 9 than at pH 4 or 7. At any pH, the degradates occurring > 10% of applied radioactivity were identified as P-NH, P-aniline-isobutyryl and P-acid. Half-lives of pyflubumide at 25 °C at pH 7 was calculated to be 27.9 days.

Photochemical degradation in buffer solutions

Photochemical degradation of pyflubumide was investigated in buffer solutions (Masaki T., 2012; E-32002) and natural water (Masaki T., 2011; E-32003).

Cylindrical test vessels (internal diameter: 46 mm, height: 30 mm) made with quartz glass (thickness: 1 mm) were capped using screw caps sealed with a Teflon-coated silicon septum after filling of the test solution. The vessels, caps and septum were sterilized before use. Test solutions were kept under sterilized conditions during irradiation.

Eight mL of phosphate buffer solution and 0.8 µg eq. of each radiolabeled pyflubumide dissolved in 40 µL acetonitrile were pipetted into sterilized test vessels to make a solution of 0.01 mg/L. Final concentration of acetonitrile in the test solution was 0.5%. Test solutions were irradiated with a Xenon arch lamp (average energy: 3.54 MJ m^{-2} d^{-1}) at a distance of 25 cm. UV shorter than 290 nm wavelength irradiated by Xenon arch lamp was filtered off. Test vessels were regularly moved to ensure uniform irradiation.

Dark control vessels were prepared by wrapping vessels with aluminum foil. Dark control sample was incubated under the same condition as the irradiation samples.

At each sampling time, duplicate test vessels were collected. Immediately after sampling, test vessels were connected to a urethane tube (3.2 cm^3), subsequent to two glass test tubes filled with 5 mL of 20% ethanolamine through Teflon-coat silicon septum. Volatile radioactivity was introduced into the connected traps through purging N_2 gas for about 10 min. Then, test solution was transferred into glass test tubes.

The whole volume of test solution collected from the test vessel was extracted twice with 4 mL ethyl acetate. If more than 5% of applied radioactivity were determined in the aqueous phase, the test solution was extracted again with equivalent volume of acetonitrile after addition of 1/10 volume 1M HCl and 1.54 g ammonium sulfate (saturated condition). Both extracts were combined for determination of radioactivity and subsequent chromatographic analysis. Ethanolamine traps were directly subjected to radioactive determination. Radioactivity absorbed in urethane traps was extracted with acetone for determination of radioactivity.

Aliquots of concentrated extract and reference standard were applied to TLC plates for two-dimensional chromatography.

Selected extracts were subjected to HPLC analysis. Samples for injection were prepared by concentration and reconstitution with acetonitrile/water (1/1). Identities of radioactive components were confirmed by comparison with UV absorbance of co-chromatographed reference substances.

Radioactivity trapped in 20% ethanolamine sampled after 30 days irradiation was released by titration of 5M HCl and introduced into 1M NaOH trap with N_2 gas purge. An aliquot of 1M NaOH trap was mixed with 1M $BaCl_2$ to confirm formation of insoluble precipitation. Ethanolamine, 1M NaOH and supernatant after $BaCl_2$ precipitation were also subjected to radioactive determination.

The degradation rate constants and half-lives were determined in the same manner as for hydrolytic degradation. Further, degradation specific to irradiation was also determined.

The degradation half-life under natural sunlight in Tokyo (north latitude of 35 degrees) from April until June was also determined using the energy of the Xenon ark lamp.

Phenyl-label

In the irradiated samples, extracted radioactivity with ethyl acetate and acetonitrile in the presence of saturated ammonium sulfate accounted for 73-102% AR. Radioactivity remaining in the aqueous phase was < 3.4% AR.

Chromatographic analysis indicated rapid degradation of phenyl-labeled pyflubumide under irradiation. The parent compound accounted for 99% of AR on day 0 but significantly decreased to 0.1% AR on day 14. Major degradates in the extracts were P-NH and P-aniline-isobutyryl, at the maximum at 31% on day 2 and 47% on day 6. In addition, several minor radioactive components including P-NH-1-H, P-aniline and unknown degradates was detected with maximum amounts of less than 6.7% of AR. Radioactivity remained at the TLC origin after development accounted for 3.7% even at maximum value. Volatile radioactivity recovered from ethanolamine and urethane traps was 18% and 1.8% AR after 30 days of irradiation.

In the dark controls, radioactivity extracted with ethyl acetate and acetonitrile in the presence of saturated ammonium sulfate accounted for 99-102% AR. Radioactivity remained in aqueous phase was < 1.1% AR. Results of the chromatographic analysis indicated moderate degradation of pyflubumide under dark condition. Radioactivity assigned to the parent compound declined to 43% until day 30. Resultant major degradates were P-NH and P-aniline-isobutyryl of which maximum radioactivity was 38% and 18% AR on day 30, respectively.

Table 13a Photolytic degradation of phenyl-labelled pyflubumide-Irradiated

Fraction	% of Applied radioactivity (at specified days after treatment)							
Component	0	1	3	4	6	10	14	30
Extracts (ethyl acetate, acetonitrile)	100	102	99	90	91	90	83	73
Pyflubumide	99	48	22	5.7	1.4	0.3	0.1	0.1
P-NH	0.6	22	31	29	25	9.0	6.6	1.9
P-NH-1-H	-	0.2	0.9	1.6	1.5	1.9	1.5	1.4
P-aniline-isobutyryl	0.3	29	37	42	47	42	37	20
P-aniline	-	0.3	0.6	1.0	1.7	1.8	1.6	1.3
Unknown (U2)	-	0.6	1.4	2.1	3.1	6.0	6.7	6.7
Unknown (U13)	-	-	1.2	1.1	2.3	2.2	5.6	4.9
Others*	-	1.6	4.7	6.9	8.8	24	22	33
TLC Origin	-	-	-	-	-	2.0	1.8	3.7
Ethanolamine trap	N.A.	-	1.2	2.1	1.2	6.2	13	18
Urethane trap	N.A.	0.3	0.4	0.5	0.3	1.1	1.2	1.8
Aqueous phase	0.1	1.3	2.2	2.1	3.4	0.2	0.3	0.8
Total	100	103	103	95	96	98	97	94

Table 13b Photolytic degradation of phenyl-labelled pyflubumide-Dark Control

Fraction	% of Applied radioactivity (at specified days after treatment)			
Component	3	7	14	30
Extracts (ethyl acetate, acetonitrile)	102	101	100	99
Pyflubumide	87	83	75	43
P-NH	11	14	19	38
P-NH-1-H	-	-	-	-
P-aniline-isobutyryl	4.3	4.0	6.2	18
P-aniline	-	-	-	-
Unknown (U2)	-	-	-	-
Unknown (U13)	-	-	-	-
Others*	-	-	-	-
TLC Origin	-	-	-	-
Ethanolamine trap	N.A.	N.A.	0.7	0.2
Urethane trap	N.A.	N.A.	0.8	0.4
Aqueous Phase	0.5	0.2	1.1	0.9
Total	103	101	103	100

– Not analysed

N.A. Not applicable

* Sum of unknown radioactivity accounting to below 4.7% of applied for each component

Pyrazole-label

In the irradiated samples, extracted radioactivity with ethyl acetate and acetonitrile in the presence of saturated ammonium sulfate accounted for 91-98% AR. Radioactivity remaining in the aqueous phase was < 2.0% AR.

Chromatographic analysis indicated rapid degradation of pyrazole-labelled pyflubumide under irradiation. The parent accounted for 97% AR on day 0 but decreased markedly to 2.3% AR at day 10. Major degradates in the extracts were P-NH and P-acid, of which maximum accountability was 40% AR on day 6 and 59% AR on day 14, respectively. P-amide, the compound without aniline moiety, accounted for 12% AR after 30 days of irradiation. Several minor radioactive compounds, including P-NH-1-H, P-acid-1-H and unknowns, were detected with maximum amounts < 5.2% of AR. Radioactivity remained at TLC origin after development was 10% on day 30. This polar radioactivity was separated into three components each less than 5.3% of AR when a polar solvent system was used. Volatile radioactivity recovered from ethanolamine and urethane traps was 2.4% and 0.3% at the maximum.

In the dark controls, radioactivity extracted with ethyl acetate and acetonitrile in the presence of saturated ammonium sulfate accounted for 100-101%. Radioactivity remaining in the aqueous phase was < 1.6% of AR. Chromatographic analysis indicated moderate degradation of pyrazole-labelled pyflubumide under dark conditions. The parent compound declined to 68% until day 30. Major degradates were P-NH, of which at the maximum accounted for 29% on day 30.

Table 14 a Photolytic degradation of pyrazole-labelled pyflubumide-Irradiated

Fraction	% of Applied radioactivity (at specified days after treatment)							
Component	0	1	2	4	6	10	14	30
Extracts (ethyl acetate, acetonitrile)	98	95	95	95	97	97	98	91
Pyflubumide	97	50	24	12	3.3	2.3	6.5	0.4
P-NH	1.0	24	33	32	40	25	15	1.1
P-NH-1-H	-	-	2.7	2.9	2.7	3.7	2.7	0.9

Fraction	% of Applied radioactivity (at specified days after treatment)							
Component	0	1	2	4	6	10	14	30
P-acid	-	21	35	44	48	50	59	49
P-acid-1-H	-	-	0.2	0.6	0.8	1.8	0.7	2.7
P-Amide	-	-	1.0	1.9	2.0	6.8	4.5	12
Others*	-	-	-	-	-	2.5	5.1	14
TLC Origin	-	-	-	1.3	1.0	4.4	4.5	10
Ethanolamine trap	N.A.	0.1	0.5	0.5	0.5	1.1	1.7	2.4
Urethane trap	N.A.	0.3	0.2	N.D.	N.D.	0.1	0.1	N.D.
Aqueous phase	0.9	0.8	2.0	1.2	0.6	1.2	0.0	1.2
Total	99	97	98	97	98	99	100	94

Table 14 b Photolytic degradation of pyrazole-labelled pyflubumide – Dark Control

Fraction	% of Applied radioactivity (at specified days after treatment)			
Component	3	7	14	30
Extracts (ethyl acetate, acetonitrile)	101	101	100	100
Pyflubumide	94	82	72	68
P-NH	6.2	16	24	29
P-NH-1-H	-	-	-	-
P-acid	0.8	2.8	4.2	3.5
P-acid-1-H	-	-	-	-
P-Amide	-	-	-	-
Others*	-	-	-	-
TLC Origin	-	-	-	-
Ethanolamine trap	N.A.	N.A.	1.4	N.D.
Urethane trap	N.A.	N.A.	0.3	0.3
Aqueous phase	0.5	1.3	1.6	1.6
Total	101	102	103	102

- Not analysed

N.A Not applicable

* Sum of unknown radioactivity accounting to below 5.2% of applied for each component

Assuming that photodegradation of pyflubumide in buffer solution to be a pseudo-first order reaction from the linearity, the calculated mean daily photodegradation rate constants of pyflubumide under artificial sunlight at pH 4 were 0.62. Degradation half-life was 1.2 days.

The photodegradation rate constant, specific to irradiation determined by subtraction of dark condition photolysis, was 0.60. Half-life based on this rate constant was 1.2 days.

Based on the cumulative energy of xenon lamp necessary to achieve half degradation of the test substance (IDT_{50}) were 3.5 and 4.7 ($MJ/m^2/d$) for each label, which are equivalent to 5.2 and 7.0 days irradiation with natural sunlight (0.674 $MJ/m^2/d$), respectively. Therefore, estimated half-lives under natural sunlight irradiation at Tokyo (north latitude of 35 degrees) in spring (from April to June) were 5.2 and 7.0 days, respectively.

The above results indicated that pyflubumide was rapidly degraded by irradiation in aqueous solutions, and ultimately mineralized through many degradates

Photochemical degradation in natural water

Natural water was obtained from Ishikawa river at a camp site in Osaka Prefecture, Japan. The collected water was filtered for sterilization using a membrane filter and placed in refrigerated storage until. The pH of the water was 6.7.

Photodegradation of radio-labelled pyflubumide in the natural water was investigated using the similar system as used for photodegradation in buffer solutions. Temperature of test solution during the incubation was controlled by tap water circulating in water bath incubating the test vessels, thermostatically controlled by the thermo regulator. The temperature of circulated tap water was continuously monitored. Targeted temperature of the test system was 25 ± 1 °C.

Phenyl-label

For the irradiated samples, radioactivity extracted with ethyl acetate and acetonitrile in the presence of saturated ammonium sulfate accounted for 65-102% AR. Radioactivity remaining in the aqueous phase was < 6.1% AR. Chromatographic analysis of extracts indicated rapid degradation of phenyl-label under irradiation. The parent compound markedly decreased to 32.5% on day 1 and 0.2% on day 14. Major degradates in extracts were P-NH and P-aniline-isobutyryl with maximum accountabilities of 35% AR on day 2 and 48% on day 4, respectively. Several minor radioactive compounds, including P-NH-1-H, P-aniline and unknowns, were detected. The maximum radioactivity of those degradates was less than 8.6% AR. Radioactivity remained at the TLC origin was 3.8% at its maximum. Volatile radioactivity recovered from ethanolamine and urethane traps was 12% and 0.5%, respectively, until 30 days irradiation.

In the dark controls, radioactivity extracted with ethyl acetate and acetonitrile in the presence of saturated ammonium sulfate accounted for 97–102% AR. Radioactivity remaining in the aqueous phase was < 2.5% AR. Chromatographic analysis of extracts indicated moderate degradation of phenyl-label under dark condition. The parent compound decreased to 60% AR on day 3 and 5.0% AR on day 30. Major degradates were assigned to P-NH and P-aniline-isobutyryl of which maximum radioactivity accounting for 38% and 54% AR, respectively on day 30.

Pyrazole-label

In the irradiated samples, extracted radioactivity with ethyl acetate and acetonitrile in the presence of saturated ammonium sulfate accounted for 89–99% AR. Radioactivity remaining in the aqueous phase was < 4.3% AR. Chromatographic analysis of extracts indicated rapid degradation of pyrazole-labelled pyflubumide under irradiation. The parent compound accounted for 38% AR on day 1 and 0.2% AR on day 14. Major degradates in the extracts were P-NH, P-acid and P-amide with maximum accountabilities of 31%, 54% and 11% on day 4, 6 and 30, respectively. Several minor radioactive compounds, including P-NH-1-H, P-acid-1-H and unknowns, were detected with a maximum total radioactivity of those degradates of less than 5.5% AR throughout the study period. Radioactivity remaining at the TLC origin was 11% AR on day 30. This radioactivity was separated into 4 minor components each below 5% AR on TLC chromatogram using a polar solvent system. Volatile radioactivity recovered from ethanolamine trap accounted for 2.2% AR at its maximum.

In the dark controls, radioactivity extracted with ethyl acetate and acetonitrile in the presence of saturated ammonium sulfate accounted for 94-100%. Radioactivity remaining in the aqueous phase was < 1.7% AR. Chromatographic analysis indicated moderate degradation of pyflubumide under dark conditions. The parent compound decreased to 66% on day 3 and 23% on day 30. Major degradates were P-NH, P-Acid, of which maximum radioactivity was 58% and 16%, respectively on day 30.

Radioactivity trapped in 20% ethanolamine collected after 30 days irradiation of phenyl-label was characterized. Addition of 1M $BaCl_2$ into 1M NaOH solution resulted in formation of radioactive precipitation due to $BaCO_3$ formation. The result indicated radioactivity trapped in 20% ethanolamine was derived from CO_2 produced through photodegradation of the test substances.

Daily photolysis rate constants of phenyl- and pyrazole-labelled pyflubumide under artificial sunlight were 0.76–0.77. Degradation half-lives thus obtained were 0.9 days.

At dark conditions, daily photolysis rate constants were 0.04–0.16; therefore, photolysis-specific rate constant determined by subtraction of rate constant on dark condition was 0.61–0.72. Half-lives based on the photolysis-specific rate constant were 1.0–1.1 days.

Estimated half-lives under natural sunlight irradiation in Tokyo (Lat. 35 °N) in spring were 5.1–6.0 days, respectively for pyrazole- and phenyl-labels.

Summary of hydrolysis and photolysis

Pyflubumide was significantly hydrolysed at all tested pH values and temperatures with a faster hydrolysis at pH 9 than pH 4 or pH 7. Estimated half-lives at 20 °C are 41.7 days at pH 4, 37.4 days at pH 7 and 15.1 days at pH 9. Regardless of pH, major hydrolysates which occur at > 10% AR were P-NH, P-aniline-isobutyryl and P-acid.

In irradiated buffer solutions, pyflubumide was rapidly decomposed with mean half-life of 1.2 days compared to that of about 34 days in the dark controls. Therefore, irradiation was regarded to be one of significant factor contributing to environmental degradation of the compound. Major degradates occurring > 10% were P-NH, P-aniline-isobutyryl, P-acid and P-amide.

In irradiated natural water, decomposition was also rapid with mean half-life of 0.9 day. Pyflubumide also degraded in the dark with half-lives of 4.4 and 18.0 days respectively of phenyl- and pyrazole-label. The degradates occurring > 10% AR were the same as for in buffer solutions.

The overall proposed degradation pathway in aqueous systems is presented below.

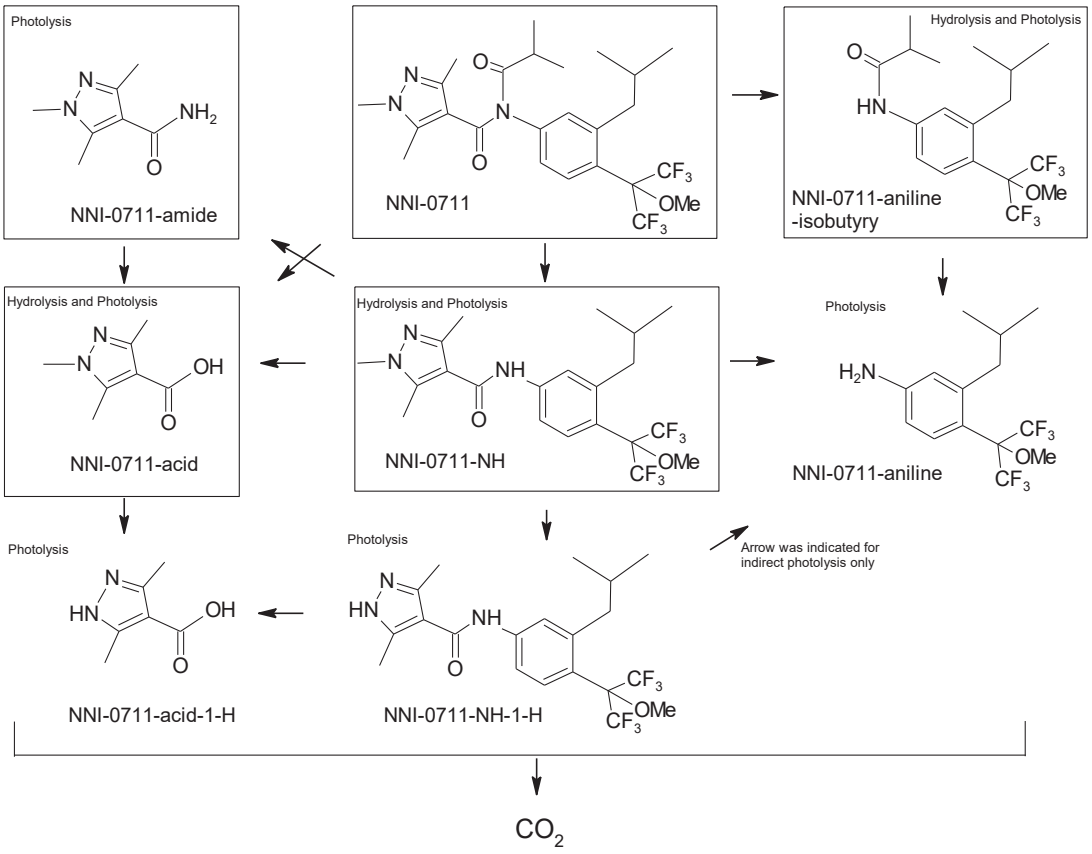


Figure 2 Proposed pathway of degradation by hydrolysis and photolysis (in buffer and natural water)(supplied by the manufacture) Degradates >10% AR are framed

Aerobic degradation in soil (Masaki T., 2011 amended 2012; E-32004)

A clay loam (sand 56.8%, silt 24.5%, and clay 18.7%; pH 6.6; organic matter 2.47; organic carbon 1.43%; maximum water holding capacity 594 g/kg) was taken from the Kochi Experiment Station of Japan Plant Protection Association and passed through a 4.75 mm sieve.

Degradation was investigated in 30 g soil aliquots (dry weight) packed into a container at a layer of approximately 1.5 cm thickness. Soil moisture was adjusted to 60% of maximum water holding capacity at the time of the soil packing. Distilled water was supplied to maintain moisture content at 40-60% of maximum water holding capacity throughout the study period. Prior to application, the test soil packed into the test containers was kept for 2 weeks at 25 ± 2 °C under dark conditions to pre-incubate.

Acetone solution of each test substance was applied at an equivalent 0.028 mg or 0.029 mg per test vessel respectively and then incorporated into the test soils using a glass rod. To collect carbon dioxide evolved in the test system, a trapping tube was attached to the top of each glass vessel packed with the soil. Incubation was performed at 25 ± 2 °C under dark conditions.

At each sampling interval, the total amount of the soil taken from a test vessel was used as a single sample. Immediately after sampling, soil weights were measured and the soil moisture contents were confirmed. Before soil sampling, nitrogen gas was introduced into the trapping tube from the nitrogen gas inlet to trap vapor phase radioactivity and the trapping tube was then removed from the vessel.

Shaking extractions of the radioactivity in soil were repeated 3 times with 150 mL of acetone for 15 minutes and repeated further 2 times with 150 mL of acetone/water (4/1) and of acetone/0.1M HCl (4/1). Each extract was refrigerated before the separate determination of the radioactivity by LSC. The PES were air dried and used for the determination of unextracted radioactivity. The limit of quantification for determination of radioactive concentration in soil was 0.4–0.7% AR.

The total amount of soda lime recovered from each trapping tube was transferred to a side-arm flask and neutralized with a few drops of 4 N or 6 N HCl under nitrogen stream and evolved gases during the course were trapped with triplicate 20% ethanolamine. The trapping liquids and soda lime neutralizing solution were mixed with liquid scintillator and then provided for determination of radioactivity. In order to qualify the trapping radioactivity, collected ethanolamine solution was neutralized with titration of 1M sulfuric acid under nitrogen stream as stated above and evolved gases during the course were collected with triplicate 1M KOH traps. An aliquot of 1M KOH solution was provided for determination of radioactivity and aliquots were mixed with saturated $BaCl_2$ solution to confirm formation of insoluble salts with Ba^{2+}. Radioactivity in generated precipitation was measured for confirmation that the radioactivity in the traps was derived from carbon dioxide.

Urethane traps recovered from the trapping tubes were filled in plastic syringes and extracted 2 times with 4 mL of acetone and the radioactivity in extracts obtained was determined. PES from this procedure were air-dried, weighed and aliquots of the residues were taken to combust by sample oxidizer and evolved carbon dioxide was then collected and the radioactivity determined. Air dried solids were homogeneously mixed with an equivalent amount of cellulose powder to optimize combustion efficiency.

The soil extracts containing radioactivity recovered > 5% AR were used for separation and quantification of degradates. Each radioactivity was quantified using TLC-RLG (thin-layer chromatography-radio-luminography) and identified by using both TLC-RLG and RI-HPLC. In the case of a very low radioactivity in the samples, the soil extracts were concentrated. After concentration of each soil extract an aliquot of the concentrate was spotted on TLC plate along with standard substances for 2-dimension chromatography. To identify degradation products an aliquot of the soil extracts was subjected to HPLC analysis.

The acetone extract contained 90-105% AR. Radioactivity extracted with acetone/distilled water (4/1, v/v) and acetone/0.1M HCl (4/1, v/v) were at a maximum 3.5% and 3.3% AR, respectively and unextracted radioactivity was only up to 2.6%. AR up to 1.0% and 0.1% AR were recovered in the soda lime traps from the pyrazole and phenyl-labelled pyflubumide 180 days after treatment, respectively. No radioactivity was observed in the urethane traps of both labels.

The acetone extracts were analysed for the distribution of parent and degradates by TLC and RI-HPLC. The distribution is shown in the table below. The parent pyflubumide was found with 105% of applied radioactivity immediately after treatment and decreased rapidly to 33-35% AR on day 180, for the both labels. Elimination of an isobutyryl group from the parent molecule resulted in formation of P-NH as a main metabolite to reach up to 82% of applied radioactivity (pyrazole-label after 112 days).

P-NH-5-CH_2OH was detected up to 6.6% AR (pyrazole-label after 180 days) and P-NH-RfOH, P-NH-3-CH_2OH, P-aniline-isobutyryl and P-acid were detected at ≤ 1.5% AR.

Degradation of pyflubumide in sterilized soil was obvious and P-NH formed at levels of 60% AR for both labels. However, the formation level was lower than in non-sterilized soil, indicating that microorganisms may degrade pyflubumide to P-NH. In a sterilized soil the hydrolysates P-acid and P-aniline-isobutyryl formed 2.6% and 5.4% of applied radioactivity, respectively.

Table 15 Radioactive compounds after radio-labelled pyflubumide was applied to soil

Phenyl-label

Fraction	% of Applied radioactivity (at specified days after treatment)			
Component	28	56	180	180*
Extraction with acetone	97	96	92	99
Pyflubumide	37	19	5.0	33
P-NH	57	72	77	60
P-NH-RfOH	0.2	0.5	1.1	N.D.
P-NH-5-CH_2OH	0.7	2.3	6.0	N.D.
P-NH-3-CH_2OH	N.D.	0.2	0.9	N.D.
P-aniline-isobutyryl	1.3	1.5	0.8	5.4
P-acid	-	-	-	-
Others	N.D.	0.7	2.0	N.D.
Extraction with acetone/water	1.5	2.0	3.4	1.6
Extraction with acetone/0.1M HCl	1.3	2.1	3.3	1.1
Trap with soda lime	N.D.	N.D.	0.1	-
Unextracted	0.7	0.9	2.6	0.7
Total	100	102	102	102

Pyrazole-label

Fraction	% of Applied radioactivity (at specified days after treatment)								
Component	0	1	3	14	28	56	112	180	180*
Extraction with acetone	105	97	96	96	98	95	94	90	98
Pyflubumide	105	95	88	52	40	20	6.6	4.0	35
P-NH	0.4	2.6	8.1	43	55	71	82	75	60
P-NH-RfOH	N.D.	N.D.	N.D.	0.1	0.3	0.7	0.9	1.5	N.D.
P-NH-5-CH_2OH	N.D.	N.D.	N.D.	0.5	1.1	2.3	2.9	6.6	N.D.
P-NH-3-CH_2OH	N.D.	N.D.	N.D.	0.1	0.2	0.4	0.4	0.7	N.D.
P-aniline-isobutyryl	-	-	-	-	-	-	-	-	-
P-acid	N.D.	0.1	0.1	0.4	0.4	0.2	0.2	0.2	2.6

Fraction	% of Applied radioactivity (at specified days after treatment)								
Component	0	1	3	14	28	56	112	180	180*
Others	N.D.	N.D.	N.D.	N.D.	0.4	1.1	1.4	2.2	N.D.
Extraction with acetone/ water	-	0.7	1.0	1.6	1.7	2.1	2.3	3.5	1.9
Extraction with acetone/0.1M HCl	-	0.4	0.7	1.3	1.5	2.0	2.3	2.9	1.5
Trap with soda lime	-	N.D.	N.D.	N.D.	0.1	0.4	0.5	1.0	-
Unextracted	0.3	0.4	0.4	0.6	0.7	1.0	1.2	2.2	1.3
Total	106	99	98	99	102	101	101	100	102

– Not analysed

* Sterilized soil

During the test period, pyflubumide disappeared according to a pseudo-first-order reaction process and its DT_{50} was calculated from the degradation rate constant (k = –0.019) of pyrazole-label to be 37.2 days.

The proposed degradation pathway in aerobic soil is shown below.

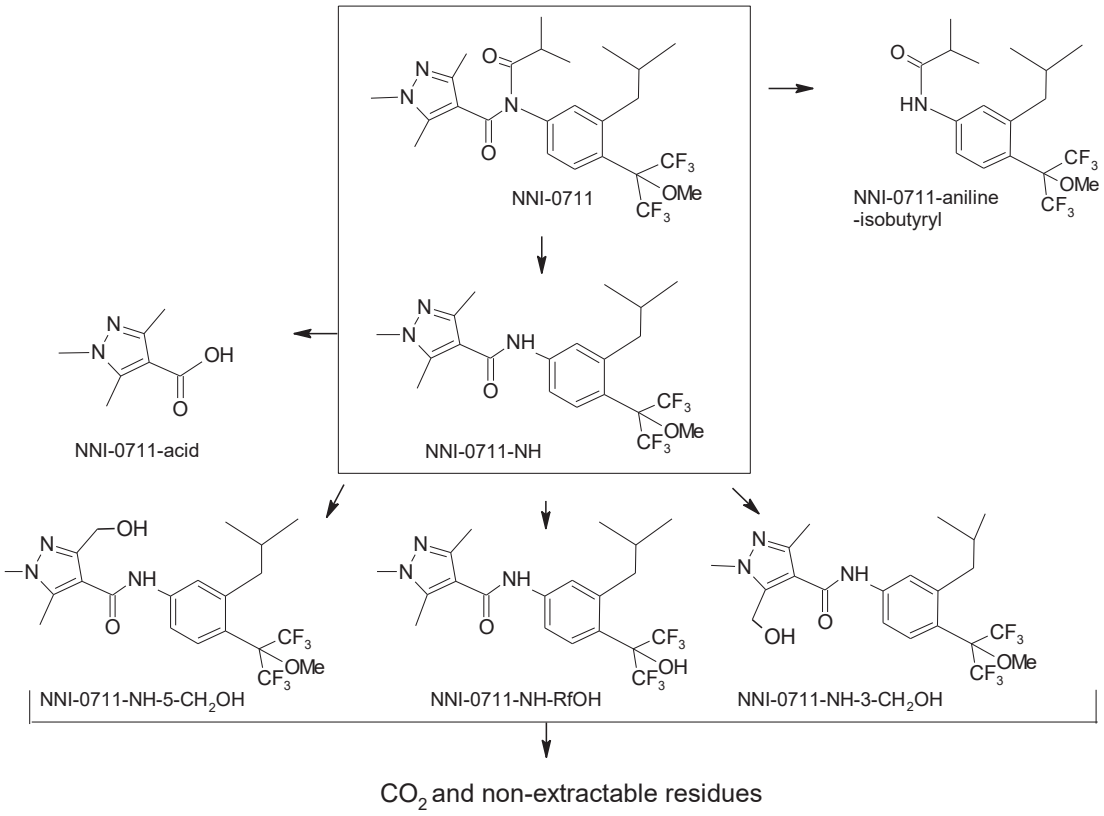


Figure 3 Proposed pathway of aerobic degradation in soil (supplied by the manufacturer)

Degradates >10% AR are framed

Summary of aerobic degradation in soil

Pyflubumide degraded in soil under laboratory conditions with a half-life of 37.2 days. The main degradate formed was P-NH by eliminating the isobutyryl group and it reached up to 82% AR after

112 days. Consequential mineralization to carbon dioxide was confirmed and a small amount of unextracted radioactivity existed. P-NH was the only degradate found above 10% AR.

Residues in Succeeding or Rotational Crops

As apple and tea, for which the supervised residue trials were provided for MRL setting to the current Meeting, are both permanent crops, no succeeding or rotational crop studies were provided to the Meeting.

METHODS OF RESIDUE ANALYSIS

The Meeting received information on analytical methods together with validation data for residues of pyflubumide in plant matrices.

Analytical Methods for Determination of Pyflubumide Residues

Analytical method for data generation and for enforcement

Method A

Analyte: Pyflubumide, P-NH, P-acid, P-aniline-isobutyryl

Matrix: Apple, processed apple commodities; and dry tea leaves and tea infusion

Description: Sample preparation: Dry tea leaves are pulverized and soaked in water. Apple samples are homogenized. Tea infusion samples are generated by extraction with boiling water and subsequently filtered. Infusion samples were cleaned-up using a disk cartridge and then graphite carbon mini-columns.

Extraction: Pyflubumide, P-NH and P-aniline-isobutyryl are extracted from the above matrices with acetone 1 to 3 times and partitioned with hexane/saturated sodium chloride aqueous solution. The extracts are cleaned up by graphite carbon mini columns with hexane or by dehydration and re-dissolving the extract in acetone. For P-acid, partitioning is with ethyl acetate and the extracts are cleaned-up by an anion-exchange mini-column. After clean-up, the eluates are concentrated.

Separation and Detection: The concentrates of all samples are dissolved in methanol/water, acetone or acetonitrile (comparable polarity index P = 5.1-5.8) and analysed by HPLC-MS or HPLC-MS/MS, expect P-aniline-isobutyryl is analysed by HPLC-MS.

HPLC uses a C_{18} column with mobile phase of methanol or acetonitrile (comparable polarity index P = 5.1-5.8) /0.1% acetic acid (or formic acid) as mobile phase (for HPL-MS/MS) or acetonitrile/0.1% formic acid (HPLC-MS).

MS/MS employs electrospray ionization (ESI) in positive mode with *m/z* 536.2→155.1 for pyflubumide, *m/z* 466.2→137.1 or 111.1 for P-NH, *m/z* 400.0→343.9 for P-aniline-isobutyryl and *m/z* 154.9→111.1 for P-acid. MS employed ESI in positive mode detecting the molecular weight of each analyte.

Validation data for Method A are summarized in Tables 16 and 17 below.

Table 16 Summary of method validation of Method A for apple and tea commodities

Analyte	Matrix	Detection *m/z (MS/MS) or* m (MS)	Fortification, mg/kg	No of test	Recovery, %		RSD, %
					Range	Mean	
Apple (for data generation)(Iijima K., 2010a, R-32014)							
Pyflubumide	Fruit	MS/MS *536.2→155.1*	0.01	6	107-111	110	1.4
			1.0	6	96-103	99	2.7
P-NH	Fruit	MS/MS *466.1→137.1*	0.01	6	73-110	93	18.4
			1.0	6	78-97	87	10.5
Apple (for data generation)(Iijima K., 2010B, R-32059)							
Pyflubumide	Fruit	MS/MS *536.2→155.1*	0.01	6	99-112	105	4.6
			1.0	6	100-114	106	5.9
P-NH	Fruit	MS/MS *466.1→137.1*	0.01	6	88-105	92	7.1
			1.0	6	89–94	91	1.8
Apple (for data generation)(Takahashi Y., 2018, R-32091)							
Pyflubumide	Fruit	MS *536.2*	0.01	5	79-86	83	3.0
			0.05	5	84-87	86	1.3
			1.0	5	92-95	93	1.2
P-NH	Fruit	MS *466.2*	0.01	5	86-90	88	1.9
			0.5	5	80-87	84	3.2
P-acid	Fruit	MS/MS *155.1→111.1*	0.01	6	81-91	87	4.8
			0.5	6	77-88	83	5.5
P-aniline-isobutyryl	Fruit	MS *400.0*	0.01	6	96-101	100	1.9
			0.5	6	92-99	95	3.0
Apple (for data generation)(Nishida K., 2018, R-32092)							
Pyflubumide	Fruit	MS *536.2*	0.01	5	79-86	83	3.0
			0.5	5	84-87	86	1.3
P-NH	Fruit	MS *466.2*	0.01	5	86-90	88	1.9
			0.5	5	80-87	84	3.2
Apple (for data generation)(Kondo K., 2018, R-32093)							
Pyflubumide	Fruit	MS/MS *536.1→155.0*	0.005	5	89–95	91	3.0
			0.5	5	87-91	89	2.0
			2	5	83-90	87	3.0
P-NH	Fruit	MS/MS *466.0→137.0*	0.005	5	89–97	92	4.0
			0.5	5	90-94	92	2.0

Analyte	Matrix	Detection *m/z (MS/MS) or* m (MS)	Fortification, mg/kg	No of test	Recovery, %		RSD, %
					Range	Mean	
P-aniline-isobutyryl	Fruit	MS/MS *400.0→343.9*	0.005	5	87-94	90	3.0
			0.5	5	88-90	89	1.0
P-acid	Fruit	MS/MS *154.9→111.1*	0.005	5	63-83	76	11.0
			0.5	5	85-88	86	2.0
Processed commodities of apple (for data generation)(Kondo K., 2018, R-32093)							
Pyflubumide	Juice (clarified)	MS/MS *536.1→155.0*	0.0005	5	97-101	99	1.0
			0.05	5	88-91	90	1.0
P-NH	Juice (clarified)	MS/MS *466.0→137.0*	0.0005	5	106-144	119	14.0
			0.05	5	92-96	94	2.0
P-aniline-isobutyryl	Juice (clarified)	MS/MS *400.0→343.9*	0.0005	5	93-102	96	4.0
			0.05	5	88-96	91	3.0
P-acid	Juice (clarified)	MS/MS *154.9→111.1*	0.0005	5	70-75	72	3.0
			0.05	5	79–92	87	7.0
Pyflubumide	Wet pomace	MS/MS *536.2→155.1*	0.005	5	90-92	91	1.0
			0.5	5	89–94	91	2.0
			2.0	5	77-100	84	11
P-NH	Wet pomace	MS/MS *466.1→137.1*	0.005	5	84-96	92	5
			0.5	5	94-98	96	1
P-aniline-isobutyryl	Wet pomace	MS/MS *400.0→343.9*	0.005	5	82-87	84	3.0
			0.5	5	93-99	96	3.0
P-acid	Wet pomace	MS/MS *154.9→111.1*	0.005	5	70-75	88	10.0
			0.5	5	79–92	88	3.0
Pyflubumide	Dry pomace	MS/MS *536.2→155.1*	0.005	5	88-106	98	8.0
			0.5	5	92-96	94	2.0
			10.0	5	80-88	83	4.0
P-NH	Dry pomace	MS/MS *466.1→137.1*	0.005	5	104-118	110	5.0
			0.5	5	94-98	98	1.0
			2.0	5	77-84	81	4.0
P-aniline-isobutyryl	Dry pomace	MS/MS *400.0→343.9*	0.005	5	81-95	87	7.0
			0.5	5	87-90	88	1.0

Analyte	Matrix	Detection *m/z (MS/MS) or* m (MS)	Fortification, mg/kg	No of test	Recovery, %		RSD, %
					Range	Mean	
P-acid	Dry pomace	MS/MS *154.9→111.1*	0.005	5	85-100	92	6.0
			0.5	5	84-89	86	2.0
Pyflubumide	Sauce (pasteurized)	MS/MS *536.2→155.1*	0.005	5	88-92	90	2.0
			0.5	5	92-98	95	3.0
P-NH	Sauce (pasteurized)	MS/MS *466.1→137.1*	0.005	5	97-101	99	2.0
			0.5	5	88-93	91	3.0
P-aniline-isobutyryl	Sauce (pasteurized)	MS/MS *400.0→343.9*	0.005	5	92-102	98	4.0
			0.5	5	90-95	93	2.0
P-acid	Sauce (pasteurized)	MS/MS *154.9→111.1*	0.005	5	62-81	71	10.0
			0.5	5	88-92	90	2.0
Pyflubumide	Dried apple	MS/MS *536.2→155.1*	0.005	5	96-98	97	1.0
			0.5	5	87-92	90	2.0
P-NH	Dried apple	MS/MS *466.1→137.1*	0.005	5	90-100	95	5.0
			0.5	5	85-89	87	2.0
P-aniline-isobutyryl	Dried apple	MS/MS *400.0→343.9*	0.005	5	93-102	96	4.0
			0.5	5	80-88	85	3.0
P-acid	Dried apple	MS/MS *154.9→111.1*	0.005	5	75-85	77	6.0
			0.5	5	80-84	82	2.0
Apple (for enforcement; ILV)(Watanabe Y., 2018a, A-32019)							
Pyflubumide	Fruit	MS *536.2*	0.01	5	94-99	97	2.2
			1.0	5	103-105	104	1.0
P-NH	Fruit	MS *466.1*	0.01	5	87-95	91	3.9
			0.5	5	91-98	93	2.9
Tea leaves, dry (for data generation)(Odanaka Y., 2009a, R-32004)							
Pyflubumide	Dry leaves	MS/MS *536.2→155.1*	0.05	6	107-110	109	1.1
			100	6	89–95	92	2.3
P-NH	Dry leaves	MS/MS *466.1→137.1*	0.05	6	95-112	102	6.3
			100	6	89–92	90	1.3
Tea leaves, dry and infusion (for data generation)(Ihara T., 2009a, R-32006)							
Pyflubumide	Dry leaves	MS/MS *536.2→155.1*	0.05	3	73-75	74	1.4
			100	3	70-77	73	5.2

Analyte	Matrix	Detection *m/z (MS/MS) or* m (MS)	Fortification, mg/kg	No of test	Recovery, %		RSD, %
					Range	Mean	
P-NH	Dry leaves	MS/MS *466.2→111.1*	0.05	3	87-90	88	2.0
			100	3	81-88	84	4.5
Pyflubumide	Infusion	MS/MS *536.2→155.1*	0.05	3	82-89	86	4.1
			5	3	70-73	71	2.4
P-NH	Infusion	MS/MS *466.2→111.1*	0.05	3	90-96	92	3.8
			5	3	71-79	75	5.4
Tea leaves, dry and infusion (for data generation)(Ihara T., 2009b, R-32007)							
Pyflubumide	Dry leaves	MS/MS *536.2→155.1*	0.05	3	73-75	74	1.4
			100	3	70-77	73	5.2
P-NH	Dry leaves	MS/MS *466.2→111.1*	0.05	3	87-90	88	2.0
			100	3	81-88	84	4.5
Pyflubumide	Infusion	MS/MS *536.2→155.1*	0.05	3	82-89	86	4.1
			5	3	70-73	71	2.4
P-NH	Infusion	MS/MS *466.1→111.1*	0.05	3	90-96	92	3.8
			5	3	71-79	75	5.4
Tea leaves, dry (for data generation)(Odanaka Y., 2009b, R-32008)							
Pyflubumide	Dry leaves	MS/MS *536.2→155.1*	0.05	6	107-119	112	4.0
			100	6	93-96	94	1.2
P-NH	Dry leaves	MS/MS *466.1→137.1*	0.05	6	98-111	104	4.5
			100	6	89–95	93	2.3
Tea leaves, dry (for data generation)(Iijima K., 2010c, R-32032)							
Pyflubumide	Dry leaves	MS/MS *536.2→155.1*	0.01	6	106-113	110	2.4
			4	3	111-117	114	2.6
			40	3	101-104	103	1.5
P-NH	Dry leaves	MS/MS *466.1→137.1*	0.01	6	96-105	100	3.5
			4	3	91-95	93	2.2
			40	3	95-102	98	3.7
Tea leaves, dry (for data generation)(Imura M., 2010, R-32034)							
Pyflubumide	Dry leaves	MS/MS *536.2→155.1*	0.01	6	80-104	91	15.3
			0.1	3	81-82	82	0.7
P-NH	Dry leaves	MS/MS *466.2→111.1*	0.01	6	68-88	77	10.2
			0.05	3	81-84	82	1.9
			0.2	3	84-86	85	1.4

Analyte	Matrix	Detection *m/z (MS/MS) or* m (MS)	Fortification, mg/kg	No of test	Recovery, %		RSD, %
					Range	Mean	
Tea leaves, dry and infusion (for data generation)(Ihara T., 2009b, R-32071)							
Pyflubumide	Dry leaves	MS/MS *536.2→155.1*	0.01	2	80-101	90	N/A
			5	1	92	92	N/A
			10	1	89	89	N/A
P-NH	Dry leaves	MS/MS *466.2→111.1*	0.01	2	71-72	72	N/A
			5	1	84	84	N/A
			10	1	92	92	N/A
Pyflubumide	Infusion	MS/MS *536.2→155.1*	0.01	2	101-104	102	N/A
			0.1	2	70-91	80	N/A
P-NH	Infusion	MS/MS *466.2→111.1*	0.01	2	86-97	92	N/A
			0.1	2	72-87	80	N/A
Tea leaves, dry and infusion (for enforcement, ILV)(Watanabe Y., 2018b, A-32020)							
Pyflubumide	Dry leaves	MS/MS *536.2→155.1*	0.01	5	74-76	75	1.1
			10.0	5	70-75	73	2.5
P-NH	Dry leaves	MS/MS *466.1→137.1*	0.01	5	71-71	71	0.4
			10.0	5	71-72	71	0.8
Pyflubumide	Infusion	MS/MS *536.2→155.1*	0.01	5	70-71	70	0.2
			10.0	5	70-70	70	0.2
P-NH	Infusion	MS/MS *466.1→137.1*	0.01	5	72-75	73	1.6
			10.0	5	71-73	72	1.1

Table 17 Summary of performance characteristics of Method A (including the data from ILV studies)

LOQ:	0.01 mg/kg for apple (except in one study 0.05 mg/kg), dry tea leaves & tea infusion; and 0.005 mg/kg for apple processed commodities of apple
Recovery:	Mean recoveries for all the analytes tested: 81–110% for apple fruit; 71–119% for processed apple commodities: 72–114% for dry tea leaves; and 70–92% for tea infusion
Repeatability:	RSD were < 20% for all analytes tested.
Specificity:	No interfering peaks were observed at the retention time of any of the analytes when matrix blank samples were injected.
Linearity:	Correlation coefficients were > 0.99 for all analytes tested; 1–40 ng/mL (0.001–0.04 ng) for apple; and 0.2–5 ng/mL (0.001–0.025 ng) for tea leaves and infusion.
Matrix effects:	No matrix effects were observed at the retention time of any of the analytes when matrix blank samples were injected.

Extraction efficiency was not studied for Method A as the solvent used for extraction in the plant metabolism studies and in Method A was acetone.

Method A was found suitable for data development for apple and tea.

Multi-residue method

QuEChERS method

Analyte: Pyflubumide and P-NH

Matrix Apple, grape, wheat grain, canola seed, tea

Description: Extraction: Residues of pyflubumide and P-NH are extracted from samples with HPLC-grade water/acetonitrile (1:2, v/v) and cleaned up with QuEChERS salt packets. The extracts are concentrated.

Separation and Detection: Concentrated sample extracts are diluted with acetonitrile (for apple wheat) or mixed with matrix control (for grape, canola seed, tea), and analysed by HPLC-MS/MS. HPLC uses a C_{18} column (ACE Excel 2 Phenyl 50 × 2.1 mm) with water/0.1% acetic acid and acetonitrile/0.1% acetic acid as mobile phases. MS/MS employed electrospray ionization (ESI) in positive mode with *m/z* 536.2→111.5 and 155.1 for pyflubumide, and *m/z* 466.2→111.5 and 137.6 for P-NH.

Validation data for Method A are summarized in Tables 18 and 19 below.

Table 18 Summary of method validation of QuEChERS method for various plant commodities

Analyte	Matrix	Detection m/z (MS/MS) or m (MS)	Fortification mg/kg	No of test	Recovery, %		RSD, %
					Range	Mean	
Apple (Feten E. and Van Middlesworth B, 2019, A-32021)							
Pyflubumide	Fruit	MS/MS 536.2→111.5	0.005	5	89-102	97	5.3
			0.05	5	103-108	106	2.0
Pyflubumide	Fruit	MS/MS 536.2→155.1	0.005	5	88-114	101	10
			0.05	5	105-114	110	3.1
P-NH	Fruit	MS/MS 466.1→111.5	0.005	5	79-106	87	13
			0.05	5	88-92	90	1.8
P-NH	Fruit	MS/MS 466.1→137.6	0.005	5	66-90	77	12
			0.05	5	79-82	81	1.6
Grapes (Feten E. and Van Middlesworth B, 2019, A-32021)							
Pyflubumide	Fruit	MS/MS 536.2→111.5	0.005	5	89–96	94	3.0
			0.05	5	94-97	96	1.2
Pyflubumide	Fruit	MS/MS 536.2→155.1	0.005	5	94-98	96	1.6
			0.05	5	94-97	95	1.2
P-NH	Fruit	MS/MS 466.1→111.5	0.005	5	92-100	97	3.1
			0.05	5	96-99	98	1.2
P-NH	Fruit	MS/MS 466.1→137.6	0.005	5	96-102	98	2.5
			0.05	5	96-100	98	1.9
Wheat (Feten E. and Van Middlesworth B, 2019, A-32021)							
Pyflubumide	Grain	MS/MS 536.2→111.5	0.005	5	94-100	98	2.5
			0.05	5	97-99	97	1.2

Analyte	Matrix	Detection m/z (MS/MS) or m (MS)	Fortification mg/kg	No of test	Recovery, %		RSD, %
					Range	Mean	
Pyflubumide	Grain	MS/MS 536.2→155.1	0.005	5	96-107	101	4.1
			0.05	5	97-98	97	0.6
P-NH	Grain	MS/MS 466.1→111.5	0.005	5	87-100	92	5.4
			0.05	5	91-95	93	1.6
P-NH	Grain	MS/MS 466.1→137.6	0.005	5	79-100	88	9.0
			0.05	5	83-85	84	1.2
Canola (Feten E. and Van Middlesworth B, 2019, A-32021)							
Pyflubumide	Seed	MS/MS 536.2→111.5	0.005	5	85-92	89	3.3
			0.05	5	87-92	89	2.4
Pyflubumide	Seed	MS/MS 536.2→155.1	0.005	5	89–94	92	2.0
			0.05	5	85-92	88	3.2
P-NH	Seed	MS/MS 466.1→111.5	0.005	5	85-96	90	5.3
			0.05	5	90-94	91	1.8
P-NH	Seed	MS/MS 466.1→137.6	0.005	5	80-90	86	4.4
			0.05	5	86-92	90	2.8
Tea (Feten E. and Van Middlesworth B, 2019, A-32021)							
Pyflubumide	Leaf	MS/MS 536.2→111.5	0.005	5	87-96	90	3.8
			0.05	5	93-100	96	2.7
Pyflubumide	Leaf	MS/MS 536.2→155.1	0.005	5	92-95	93	1.4
			0.05	5	93-100	97	3.3
P-NH	Leaf	MS/MS 466.1→111.5	0.005	5	93-98	95	2.7
			0.05	5	94-99	96	2.4
P-NH	Leaf	MS/MS 466.1→137.6	0.005	5	81-94	86	6.2
			0.05	5	92-97	94	1.9
Apple (ILV)(Whiting S., 2019, A-32022)							
Pyflubumide	Fruit	MS/MS 536.2→111.5	0.005	5	72-94	84	10
			0.05	5	90-94	93	1.9
Pyflubumide	Fruit	MS/MS 536.2→155.1	0.005	5	80-93	88	6.0
			0.05	5	89–95	93	2.5
P-NH	Fruit	MS/MS 466.1→111.5	0.005	5	72-77	74	2.8
			0.05	5	89–95	92	2.4
P-NH	Fruit	MS/MS 466.1→137.6	0.005	5	72-77	75	2.6
			0.05	5	89–94	91	2.1
Grapes (ILV)(Whiting S., 2019, A-32022)							
Pyflubumide	Fruit	MS/MS 536.2→111.5	0.005	5	91-99	95	3.1
			0.05	5	93	93	0.0
Pyflubumide	Fruit	MS/MS 536.2→155.1	0.005	5	93-100	96	2.9
			0.05	5	94-96	95	0.9

Analyte	Matrix	Detection m/z (MS/MS) or m (MS)	Fortification mg/kg	No of test	Recovery, %		RSD, %
					Range	Mean	
P-NH	Fruit	MS/MS 466.1→111.5	0.005	5	81-88	84	3.4
			0.05	5	94-96	95	0.9
P-NH	Fruit	MS/MS 466.1→137.6	0.005	5	76-87	82	4.9
			0.05	5	90-95	93	2.0
Wheat (ILV) (Whiting S., 2019, A-32022)							
Pyflubumide	Grain	MS/MS 536.2→111.5	0.005	5	88-98	93	4.1
			0.05	5	97-100	99	1.2
Pyflubumide	Grain	MS/MS 536.2→155.1	0.005	5	87-95	92	3.6
			0.05	5	96-102	100	2.5
P-NH	Grain	MS/MS 466.1→111.5	0.005	5	84-97	89	5.8
			0.05	5	93-100	97	2.6
P-NH	Grain	MS/MS 466.1→137.6	0.005	5	91-99	94	3.2
			0.05	5	95-99	97	1.8
Canola (ILV) (Whiting S., 2019, A-32022)							
Pyflubumide	Seed	MS/MS 536.2→111.5	0.005	5	73-87	83	6.7
			0.05	5	72-83	80	5.6
Pyflubumide	Seed	MS/MS 536.2→155.1	0.005	5	74-83	78	5.5
			0.05	5	73-81	78	4.0
P-NH	Seed	MS/MS 466.1→111.5	0.005	5	67-81	74	8.5
			0.05	5	77-84	81	3.4
P-NH	Seed	MS/MS 466.1→137.6	0.005	5	71-79	76	4.5
			0.05	5	85-111	93	12
Tea (ILV)((Whiting S., 2019, A-32022)							
Pyflubumide	Leaf	MS/MS 536.2→111.5	0.005	5	76-90	86	6.8
			0.05	5	87-90	88	1.2
Pyflubumide	Leaf	MS/MS 536.2→155.1	0.005	5	84-92	89	4.3
			0.05	5	86-92	90	2.8
P-NH	Leaf	MS/MS 466.1→111.5	0.005	5	81-87	84	2.7
			0.05	5	84-85	84	0.6
P-NH	Leaf	MS/MS 466.1→137.6	0.005	5	83-90	88	3.2
			0.05	5	83-89	86	2.8

Table 19 Summary of performance characteristics of QuEChERS method (including the data from ILV studies)

LOQ:	0.005 mg/kg for pyflubumide and P-NH in apple, grape, wheat grain, canola seed and tea
Recovery	Mean recoveries for all the analytes tested: 74–110% for apple fruit; 82–98% for grapes; 84–101% for wheat grain; 74–93% for canola seed; and 84–97% for tea leaves.;
Specificity	No interfering peaks were observed at the retention time of any of the analytes when matrix blank samples were injected.
Linearity	Correlation coefficients of > 0.99; at least between 0.75–30 ng/mL for all analytes

Repeatability	RSD <20% at 10 times LOQ
Matrix effect	Apple and wheat grain: No significant matrix effects (<20%) Grape, canola seed, tea: Matric effect of >20%. Therefore, matrix matched calibration curve was used.

The QuEChERS method was suitable for determining pyflubumide and P-NH in apple, grapes, wheat grain, canola seed and tea leaves in enforcement.

The extraction efficiency of the QuEChERS method was investigated by comparison of the residue levels of apples obtained in a supervised residue trial from Method A and from the QuEChERS method (Nakatsuji N., 2018, A-32018). Method A uses acetone as extraction solvent as in the plant metabolism studies while QuEChERS method uses acetonitrile. The concurrent mean recoveries of these methods at 0.01 and 1 mg/kg were 85–98% with RSD below 5.8%. The analytical results of untreated samples were <LOQ from the both analytical methods.

Table 20 Comparison of the analytical results of pyflubumide and P-NH from methods using acetone or acetonitrile for extraction (mean of 3 replicates)

Analyte	Method A Extraction with acetone	QuEChERS Extraction with acetonitrile
	mg-pyflubumide equivalents/kg	
Pyflubumide	0.37	0.37
P-NH	0.02	0.02
Sum	0.39	0.39
Ratio (result from Method A as I)	1	1

The extraction efficiency of acetonitrile used in the QuEChERS method was demonstrated to be equivalent to that of acetone used in the plant metabolism studies and Method A.

Storage Stability under Frozen Conditions

The stability of pyflubumide residues in commodities was investigated in homogenized apple and dried tea leaf samples stored under frozen conditions (-20 °C). The studies were conducted according to the guidelines of the Ministry of Agriculture, Forestry and Fisheries of Japan on the Data Requirements for Supporting Registration of Pesticides (November 2000-April 2019), which required storage stability study for each supervised residue trial. After all the samples from each supervised trial stored frozen were analysed, fortified control samples shall be analysed, i.e., after frozen storage for the same or longer storage period between the receiving date by the laboratory and extraction. Studies were not required if the received samples were analysed immediately after arrival in the laboratory.

Twenty grams of homogenized control samples were fortified at 0.5 mg/kg pyflubumide, P-NH, P-acid and P-aniline-isobutyryl and kept frozen at -20 °C. After the storage for the specified periods, the stored samples were analysed with Method A.

Results of storage stability studies in apple and dried tea leaves are summarized in Table 21. Percentage of pyflubumide and its metabolites remaining was not corrected for procedural recoveries.

Table 21 Storage stability of pyflubumide and metabolites in homogenized apple and dried tea leaves under frozen conditions at approximately -20 °C

Analyte	Matrix	Fortification mg/kg	Storage time, day*	% Remaining	%Remaining (Ave.)	Procedural recovery %
Apple (Takahashi Y., 2018, R-32091)[a/]						
Pyflubumide	Fruit	0.5	0		100	86
			87	89, 87	88	91[b/]

Analyte	Matrix	Fortification mg/kg	Storage time, day*	% Remaining	%Remaining (Ave.)	Procedural recovery %
			83	92, 88	90	93 [b/]
			75	100, 86	93	92 [b/]
P-NH		0.5	0		100	84
			87	90, 89	90	94 [b/]
			83	89, 88	89	94 [b/]
			75	93, 90	92	93 [b/]
P-acid		0.5	0		100	83
			87	89, 88	89	95 [b/]
			83	91, 87	89	97 [b/]
			75	89, 87	88	95 [b/]
P-aniline-isobutyryl		0.5	0		100	95
			87	94, 94	94	99 [b/]
			83	95, 92	94	95 [b/]
			75	95, 94	95	96 [b/]
Tea (Ihara T., 2009, R-32006 and R-32007)[c/]						
Pyflubumide	Dry	10	0		100	[d/]
	leaf		41	70, 74	72	[d/]
P-NH		10	0		100	[c/]
			41	74, 82	78	
Tea (Motohashi T., 2012, R-32071)[e/]						
Pyflubumide	Dry	0.1	0		100	[f/]
	leaf		82	102, 88	95	[f/]
			107	75,70	73	[f/]
P-NH		0.1	0		100	[f/]
			82	100, 96	98	[f/]
			107	73, 70	72	[f/]

* Longest storage period of each trial or longer. When the samples from supervised trials were analysed immediately after the arrival in the laboratory, storage stability studies were not conducted in accordance with the MAFF guidelines.

[a] Control samples obtained in the trials in Aomori, Iwate and Fukushima. Control samples obtained in Yamanashi and Nagano were not subjected to storage stability study as the treated samples were analysed immediately after arrival at the laboratory (one day after harvest).

[b] Average procedural recoveries at the fortification level of 0.1 mg/kg.

[c] Control sample obtained in the trial in Kagoshima.

[d] Average procedural recoveries at the fortification levels of 0.05 and 100 mg/kg were 73–74% for pyflubumide and 84–88% for P–NH.

[e] Control samples obtained in the trials in Saitama and Kochi.

[f] Average procedural recoveries at the fortification levels of 0.01 and 5 or 10 mg/kg were 78–92% for pyflubumide and 71–92% for P-NH.

The results showed that pyflubumide and P-NH was stable in homogenized apple and dried tea leaves for at least 107 days under frozen conditions. Samples from the supervised residue trials were analysed after frozen storage shorter than the storage periods shown in the above table.

The apple samples from the metabolism study were analysed by HPLC 224 to 274 days after their harvest. Although the resulting chromatograms were similar to the original analyses, storage stability in the extracts of apple samples from the supervised trials were examined along with those of processed samples (Kondo K., 2018, R-32093). The apple samples from three supervised trials were processed into pasteurized clarified apple juice, wet pomace, dried pomace, pasteurized apple sauce

and dried apple. After homogenization or pulverization, samples were extracted with acetone. All extracts were fortified at 0.5 mg/kg of pyflubumide or its metabolites and stored in the dark at 1–10 °C (actual temperature, 3.9–6.1 °C) until clean-up and LC-MS/MS analysis.

Results of storage stability studies in apple and dried tea leaves are summarized in Table 21. Percentage of pyflubumide and its metabolites remaining was not corrected for procedural recoveries.

Table 22 Storage stability of pyflubumide and metabolites in the extracts of apple and its processed commodities under storage at 1-10 °C (actual temperature: 4–6 °C) at the fortification level of 0.5 mg/kg (Kondo K., 2018, R-32093).

Analyte (Analytical method)	Storage time, day	% Remaining [a]	%Remaining (Ave.)	Procedural recovery, %
Apple				
Pyflubumide	82	89, 90, 90	90	89
P-NH	82	92, 92, 94	93	92
P-acid	82	83, 76, 78	79	86
P- aniline- isobutyryl	82	92, 92, 93	92	89
Pasteurized clarified apple juice				
Pyflubumide	69	91, 93, 92	92	90[b]
P-NH	69	92, 93, 93	93	94 [b]
P-acid	69	82, 81, 75	79	87 [b]
P-aniline-isobutyryl	69	91, 92, 93	92	91 [b]
Wet pomace				
Pyflubumide	72	88, 90, 90	89	91
P-NH	72	92, 94, 94	93	96
P-acid	72	82, 78, 79	80	88
P-aniline-isobutyryl	72	92, 91, 94	92	96
Dry pomace				
Pyflubumide	68	86, 86, 89	87	94
P-NH	68	88, 90, 94	91	98
P-acid	68	74, 76, 76	76	86
P-aniline-isobutyryl	68	87, 88, 90	88	88
Pasteurized apple sauce				
Pyflubumide	70	92, 94, 93	93	95
P-NH	70	94, 93, 95	94	91
P-acid	70	77, 79, 81	79	90
P-aniline-isobutyryl	70	92, 94, 94	93	93
Dried apple				
Pyflubumide	68	87, 89, 90	89	90
P-NH	68	92, 92, 89	91	87
P-acid	68	75, 73, 72	73	82
P-aniline-isobutyryl	68	86, 91, 91	89	85

[a] Results of samples obtained in three supervised trials

[b] Procedural recovery at 0.05 mg/kg.

Pyflubumide, P-NH, P-acid and P-aniline-isobutyryl were found to be stable in the acetone extracts of apple and its processed commodities at least for 82 days.

USE PATTERN

Pyflubumide is registered in Japan for use on crops including apple and tea. It is formulated as a suspension concentrate (SC) and used to control spider mites and Kanzawa spider mite.

The approved label in Japan was made available to the Meeting. For the purposes of estimating maximum residue levels, only the registered uses on the crops concerned and those confirmed by the relevant labels are recorded in Table 23 the application methods of all the uses below are foliar spray in the field.

Table 23 Registered uses of pyflubumide for the crops for which supervised trials were conducted (All uses employ foliar spray method)

Crop	Country	Conc. g ai/L or kg Form	Application				Minimum PHI, days
			Max No./crop/ season	g ai/hL min-max [a]	Water L/ha min-max [b]	g ai/ha min-max [c]	
Apple	JP	200 SC	1	10 (Dilution X2000)	2000-7000		1
Tea	JP	200 SC	1	5-10 (Dilution X2000-4000)	2000-4000		7

[a] Mandatory requirement

[b] Recommendation

[c] Not on the label

RESIDUES RESULTING FROM SUPERVISED RESIDUE TRIALS ON CROPS

Supervised trials using foliar spray of pyflubumide were conducted on the following crops: apple and tea. The results of these supervised trials are summarized in the following tables:

Group/Sub-group	Commodity	Country	Table No.
Pome fruits (FP)	Apple	Japan	24
Derived edible products of plant origin (DT)	Tea	Japan	25

In addition to the description and details of the field trials, each study report includes a summary of the analytical methods, together with the corresponding procedural recoveries, LOQ, LOD, and information on storage of samples. Duration of freezer storage between sampling and analysis were reported for all trials and were covered by the conditions of the freezer storage stability studies.

All appropriate trials are summarized and used. In the trials, where multiple analyses were conducted on a single sample, the mean value is used for estimation of maximum residue levels, STMR and HR. Where results from the same location with similar application timing and variety are reported, only the higher results were used..

When residues were not quantifiable, they are shown as below the LOQ of the relevant analytical method (e.g., < 0.01 mg/kg). Residues and application rate have generally been rounded to two significant figures or, for residues near the LOQ, to one significant figure.

Although control plots were included in the trials, control data are not reported in the following tables unless residues in control samples exceeded the LOQ. Results have not been corrected for concurrent method recoveries.

Residue values from the trials conducted according to the critical GAP were used for the estimation of maximum residue levels, STMR and HR. Those results included in the tables are underlined.

For calculating the sum of pyflubumide and P-NH, the concentration of P-NH is converted to that of the parent using the molecular weights. When the concentration of P-NH is below the LOQ, it is regarded as at the LOQ for calculation.

Pome fruits

Apple

Ten field trials were conducted in 2009, 2010 and 2017 in 7 different locations in Japan. All trials were designed as decline trials with treatments made 1, 3 and 7; 1, 3, 7 and 21; or 1, 7, 14 and 21 days before the 1st picking. Apple fruits were collected, packaged into cardboard boxes and shipped to the laboratory under cooled conditions. Samples were received in the laboratory on the next day after collection. The maximum frozen storage period was 73 days before analysis with Method A. Samples from three of these trials were used in an apple processing study to derive processing factors for the dietary exposure assessment.

In three trials, P-aniline-isobutyryl and P-acid were analysed but they were all below the LOQ.

Table 24 Residues in apples from supervised trials in Japan involving foliar application of pyflubumide (200 SC formulation)

Apple Location Year Variety Report No.	Application				DAT	Residues, mg/kg		
	Conc g ai/hL	Water L/ha	g ai/ha	No.	days	Pyflubumide	P-NH	Total [a]
GAP JP	10[b]	2000-7000[c]	[d]	1	PHI 1			
Akita 2009 Fuji R-32014	20	4500	900	1	<u>1</u>	0.65 (0.66/0.64)	0.01 (0.01/0.01)	0.67 (0.65+< 0.02)
					7	0.34 (0.36/0.33)	< 0.01 (< 0.01/< 0.01)	0.36 (0.34+< 0.02)
					14	0.21 (0.21/0.21)	< 0.01 (< 0.01/< 0.01)	0.23 (0.21+< 0.02)
					21	0.09 (0.09/0.09)	< 0.01 (< 0.01/< 0.01)	0.11 (0.09+< 0.02)
Fukushima 2009 Tsugaru R-32014	20	5000	1000	1	<u>1</u>	0.78 (0.79/0.78)	0.02 (0.02/0.02)	0.80 (0.78+0.02)
					7	0.28 (0.28/0.28)	0.01 (0.01/0.01)	0.30 (0.28+< 0.02)
					14	0.12 (0.12/0.12)	< 0.01 (< 0.01/< 0.01)	0.14 (0.12+< 0.02)
					21	0.10 (0.11/0.10)	< 0.01 (< 0.01/< 0.01)	0.12 (0.10+< 0.02)
Aomori 2010 Tsugaru R-32059	10	4500	450		<u>1</u>	0.12 (0.12/0.11)	< 0.01 (< 0.01/< 0.01)	0.14 (0.12+< 0.02)
					3	<u>0.13</u> (0.13/0.13)	< 0.01 (< 0.01/< 0.01)	<u>0.15</u> (0.13+< 0.02)
					7	0.06	< 0.01	0.08

Apple Location Year Variety Report No.	Application Conc g ai/hL	 Water L/ha	 g ai/ha	 No.	DAT days	Residues, mg/kg Pyflubumide	 P-NH	 Total [a]
GAP JP	10[b]	2000-7000[c]	[d]	1	PHI 1			
					 21	(0.06/0.06) 0.01 (0.01/0.01)	(< 0.01/< 0.01) < 0.01 (< 0.01/< 0.01)	(0.06+< 0.02) 0.03 (0.01+< 0.02)
Iwate 2010 Fuji R-32059	10	4500	450	1	1 3 7 21	0.46 (0.47/0.46) 0.32 (0.32/0.31) 0.24 (0.25/0.23) 0.10 (0.11/0.10)	0.01 (0.01/0.01) < 0.01 (< 0.01/< 0.01) < 0.01 (< 0.01/< 0.01) < 0.01 (< 0.01/< 0.01)	0.48 (0.46+0.02) 0.34 (0.32/< 0.02) 0.26 (0.24+< 0.02) 0.12 (0.10+< 0.02)
Aomori 2017 Fuji R-32091	10	4500	450	1	1 3 7	0.44 (0.44/0.43) 0.35 (0.36/0.34) 0.41 (0.42/0.40)	0.02 (0.02/0.02) 0.02 (0.02/0.02) 0.02 (0.02/0.02)	0.46 (0.44+0.02) 0.37 (0.35+0.02) 0.43 (0.41/0.02)
Iwate 2017 Fuji R-32091	10	4500	450	1	1 3 7	0.52 (0.54/0.50) 0.46 (0.49/0.44) 0.50 (0.50/0.49)	0.03 (0.03/0.03) 0.03 (0.03/0.03) 0.03 (0.03/0.03)	0.55 (0.52+0.03) 0.49 (0.46+0.03) 0.53 (0.50+0.03)
Fukushima 2017 Fuji R-32091	10	4500	450	1	1 3 7	0.42 (0.43/0.42) 0.42 (0.42/0.41) 0.45 (0.46/0.44)	0.02 (0.02/0.02) 0.02 (0.02/0.02) 0.02 (0.02/0.02)	0.44 (0.42+0.02) 0.44 (0.42+0.02) 0.47 (0.45+0.02)
Yamanashi 2017 Tsugaru R-32091	10	4500	450	1	1 3 7	0.23 (0.23/0.23) 0.12 (0.13/0.12) 0.06 (0.06/0.05)	0.02 (0.02/0.02) 0.01 (0.01/0.01) 0.01 (0.01/0.01)	0.25 (0.23+0.02) 0.14 (0.12+0.02) 0.08 (0.06+0.02)
Nagano 2017 Akibae R-32091	10	4500	450	1	1 3 7	0.34 (0.35/0.34) 0.26 (0.26/0.25) 0.27 (0.28/0.26)	0.01 (0.01/0.01) 0.01 (0.01/0.01) 0.02 (0.02/0.02)	0.36 (0.34+0.02) 0.28 (0.26+0.02) 0.29 (0.27+0.02)
Shiga 2017	10	4500	450	1	1	0.23 (0.23/0.23)	< 0.01 (< 0.01/< 0.01)	0.25 (0.23+< 0.02)

Apple Location Year Variety Report No.	Application				DAT	Residues, mg/kg		
	Conc g ai/hL	Water L/ha	g ai/ha	No.	days	Pyflubumide	P-NH	Total [a]
GAP JP	10[b]	2000- 7000[c]	[d]	1	PHI 1			
Rakuraku- Fuji R-32092					3 7	0.22 (0.22/0.21) 0.19 (0.20/0.18)	< 0.01 (< 0.01/< 0.01) < 0.01 (< 0.01/< 0.01)	0.24 (0.22+< 0.02) 0.21 (0.19+< 0.02)

[a] The sum of the average concentration of pyflubumide and the average concentration of P-NH, expressed in pyflubumide equivalents. The conversion factor from P-NH to pyflubumide is 1.15 (the M.W. of pyflubumide (535.5) divided by the M.W. of P-NH (465.4). When the concentration of P-NH is below the LOQ, the converted value was rounded up.

[b] Mandatory requirement

[c] Recommendation

[d] Not on the label

Derived edible products of plant origin (DT)

Tea

A total of eight independent supervised trials were conducted in 2009-2011 in 4 different locations in Japan. All trials were designed as decline trials, and tea leaves were collected 1, 7, 14 and 21 days after application. Fresh tea leaves were processed immediately after harvest by kneading, crumpling, secondary kneading, precise kneading and drying. Harvested fresh samples were subjected to steam heating treatment for ca. 45–60 sec. using a belt conveyer type steam heating machine for up to 2 kg of leaves. The weight of raw tea leaves before steam heating was 1000 g for each treated plot. The samples then were dried at 80 °C. The drying period for the treated samples was ca. 80 min. The tea leaves were then placed on a metal grid and spread over the shelf evenly. Every 30 min the shelves were replaced, and the tea leaves were turned. The fresh and dry weights of samples before and after drying were for the treated samples 1000 g and 245 g (plot A), 241 g (plot B) and 243 g (plot C), respectively. The dry weight of the tea leaves ranged from 23.75 to 24.5%. Samples were then packaged into plastic bags with aluminium film. Nitrogen gas was filled into the bags for deep freezing of the samples. Bags were kept under vacuum conditions (sealed) and sent to the laboratory under chilled conditions on the same day of harvest. The maximum frozen storage period was 3 days before analysis with Method A. In eight trials, the dried tea leaves were processed to infusions.

Table 25 Residues in dried tea leaves and tea infusion from supervised trials in Japan involving foliar application of pyflubumide (200 SC formulation)

Tea Location, Year Variety, GS Report No.	Application				DAT	Residues, mg/kg		
	Conc g ai/hL	Water L/ha	g ai/ha	No.	days	Pyflubumide	P-NH	Total [a]
GAP JP	5-10[b]	2000- 4000[c]	[d]	1	PHI 7			
Saitama [e] 2009 Yabukita	10	4000	400	1	1 <u>7</u>	91 (91.1/90.9) <u>23</u>	16 (16.4/16.1) 9.2	110 (91.0+18.6) <u>34</u>

Tea Location, Year Variety, GS Report No.	Application Conc g ai/hL	Water L/ha	g ai/ha	No.	DAT days	Residues, mg/kg Pyflubumide	P-NH	Total [a]
GAP JP	5-10[b]	2000-4000[c]	[d]	1	PHI 7			
1st pick R-32004					 14 21	(23.0/23.0) 1.6 (1.65/1.65) < 0.05 (< 0.05/< 0.05)	(9.27/9.20) 3.0 (3.00/2.99) 0.22 (0.23/0.22)	(23.0+10.6) 5.1 (1.65+3.45) 0.30 (< 0.05+0.25)
Saitama [e] 2009 Yabukita 1st pick R-32006	10	4000	400	1	1 7 14 21	88 (81.0/95.1) 23 (24.6/20.6) 1.5 (1.61/1.40) < 0.05 (< 0.05/< 0.05)	6.7 (6.90/6.41) 6.5 (6.97/6.06) 3.0 (2.99/2.91) 0.18 (0.22/0.15)	96 (88.0+7.66) 30 (22.6+7.50) 4.9 (1.51+3.39) 0.26 (< 0.05+0.21)
Kochi [f] 2009 Yabukita 1st pick R-32004	10	4000	400	1	1 7 14 21	52 (51.9/51.9) 12 (11.8/11.8) 3.9 (3.92/3.92) 0.10 (0.10/0.10)	12 (11.8/11.6) 4.1 (4.15/4.14) 2.6 (2.57/2.55) 0.25 (0.25/0.25)	65 (51.9+13.5) 17 (11.8+4.76) 6.9 (3.92+2.94) 0.39 (0.10+0.29)
Kochi [f] 2009 Yabukita 1st pick R-32006	10	4000	400	1	1 7 14 21	45 (52.3/38.2) 12 (12.6/11.0) 4.0 (4.10/3.92) 0.10 (0.11/0.10)	3.4 (4.01/2.89) 2.5 (2.61/2.43) 2.2 (2.43/1.98) 0.21 (0.22/0.20)	49 (45.2+3.97) 15 (11.8+2.90) 6.5 (4.01+2.53) 0.34 (0.10+0.24)
Mie [g] 2009 Yabukita 1st pick R-32007	5	4000	200	1	1 7 14 21	14 (15.0/12.5) 0.86 (0.97/0.76) < 0.05 (< 0.05/< 0.05) < 0.05 (< 0.05/< 0.05)	3.1 (3.13/3.00) 1.4 (1.58/1.32) 0.22 (0.22/0.21) 0.15 (0.15/0.15)	17 (13.8+3.52) 2.5 (0.86+1.67) 0.30 (< 0.05+0.25) 0.22 (< 0.05+0.17)
Mie [g] 2009 Yabukita 1st pick R-32008	5	4000	200	1	1 7 14 21	13 (13.4/13.4) 0.85 (0.85/0.85) < 0.05 (< 0.05/< 0.05) < 0.05	5.8 (5.79/5.77) 1.94 (1.97/1.92) 0.24 (0.25/0.24) 0.20	20 (13.4+6.65) 3.1 (0.85+2.23) 0.34 (< 0.05+0.28) 0.28

Tea Location, Year Variety, GS Report No.	Application Conc g ai/hL	Water L/ha	g ai/ha	No.	DAT days	Residues, mg/kg Pyflubumide	P-NH	Total [a]
GAP JP	5-10[b]	2000-4000[c]	[d]	1	PHI 7			
						(< 0.05/< 0.05)	(0.20/0.20)	(< 0.05+0.23)
Kagoshima [h] 2009 Okumidori 1st pick R-32007	5	4000	200	1	1	56 (58.1/54.4)	9.0 (9.45/8.51)	66 (56.2+10.3)
					7	24 (26.3/20.7)	5.4 (5.73/5.14)	30 (23.5+6.26)
					14	0.41 (0.42/0.40)	1.2 (1.20/1.13)	1.7 (0.41+1.33)
					21	< 0.05 (< 0.05/< 0.05)	0.16 (0.16/0.16)	0.23 (< 0.05+0.18)
Kagoshima [h] 2009 Okumidori R-32008	5	4000	200	1	1	58 (58.6/58.4)	15 (15.1/14.9)	76 (58.5+17.3)
					7	25 (25.3/25.2)	7.7 (7.73/7.71)	34 (25.2+8.89)
					14	0.44 (0.44/0.43)	1.2 (1.24/1.21)	1.8 (0.44+1.40)
					21	< 0.05 (< 0.05/< 0.05)	0.16 (0.16/0.16)	0.23 (< 0.05+0.18)
Saitama 2010 Yabukita 2nd pick R-32032	10	4000	400	1	7	1.7 (1.69/1.62)	1.4 (1.43/1.36)	3.3 (1.66+1.61)
					14	0.30 (0.30/0.29)	0.28 (0.28/0.27)	0.62 (0.30+0.32)
					21	0.01 (0.01/0.01)	0.05 (0.05/0.05)	0.07 (0.01+0.06)
Mie 2010 Yabukita 1st pick R-32032	10	4000	400	1	7	19 (19.8/18.9)	6.8 (6.93/6.67)	27 (19.4+7.82)
					14	2.2 (2.22/2.08)	1.4 (1.44/1.38)	3.8 (2.15+1.62)
					21	0.03 (0.03/0.03)	0.35 (0.36/0.34)	0.43 (0.03+0.40)
Saitama 2011 Sayama Midori 4th leaf R-32071	10	4000	400	1	7	6.1 (6.31/5.94)	2.6 (2.61/2.57)	9.1 (6.12+2.98)
					14	1.4 (1.45/1.44)	1.4 (1.40/1.36)	3.0 (1.44+1.59)
					21	0.14 (0.14/0.14)	0.25 (0.26/0.24)	0.43 (0.14+0.29)
Kochi 2011 Yabukita 4th leaf R-32071	10	4000	400	1	7	2.9 (2.90/2.90)	3.1 (3.21/3.07)	6.5 (2.90+3.61)
					14	0.09 (0.09/0.09)	1.3 (1.29/1.24)	1.5 (0.09+1.45)
					21	0.02 (0.02/0.02)	0.29 (0.30/0.28)	0.35 (0.02+0.33)

[a] The sum of the average concentration of pyflubumide and the average concentration of P-NH, expressed in pyflubumide equivalents. The conversion factor from P-NH to pyflubumide is 1.15 (the M.W. of pyflubumide (535.5) divided by the M.W. of P-NH (465.4). When the concentration of P-NH is below the LOQ, the converted

value was rounded up.

[b] Mandatory requirement

[c] Recommendation

[d] Not on the label

[e], [f], [g], [h] Same location and same application date

FATE OF RESIDUES IN STORAGE AND IN PROCESSING

Information and Data from Residues in Processed Commodities

The Meeting received information on hydrolysis simulating food processing and processing of grapes to processed commodities.

Hydrolysis

The hydrolysis of phenyl-labelled and pyrazole-labelled pyflubumide was investigated by incubation in sterile buffered aqueous solution under the conditions simulating pasteurization, baking/brewing/boiling and sterilization (Yaginuma S., 2019, PC-32038).

The aqueous buffer solution of phenyl-labelled and pyrazole-labelled pyflubumide at the nominal concentration of 1.0 mg/L were incubated at 90 °C (pH 4) for 20 minutes, 100 °C (pH 5) for 60 minutes or 120 °C (pH 6) for 20 minutes. For each set of conditions, the total radioactivity in each solution was determined by LSC at the end of the incubation period. Pyflubumide and its radio-labelled degradation products were identified and quantified by TLC-radio-luminography.

The overall recoveries of radioactivity from phenyl-labelled and pyrazole-labelled pyflubumide in test solutions were 94–100% at all conditions. The proportion of pyflubumide in the solutions was 71–97% of the applied radioactivity with the highest proportion at pH 4 at 90 °C for 20 minutes and the lowest at PH 5 at 100 °C for 120 min regardless of the position of label. P-NH, P-aniline-isobutyryl (only from the phenyl-label) and P-acid (only from the pyrazole-label) were identified as the radioactive residues in the solutions. P-aniline or any other degradation products were not detected (Tables 26 and 27).

Table 26 Effect of hydrolysis on pyflubumide in buffers at pH 4, 5 and 6 simulating food processing

Process simulated	Test condition			% Applied radioactivity	
	pH	Temp., °C	Time, min	Phenyl-label	Pyrazole-label
Pasteurization	4	90	20	99.7	96.9
Baking/brewing/boiling	5	100	6	95.1	95.8
Sterilization	6	120	20	97.5	94.4

Table 27 Identification of hydrolysate of radiolabelled pyflubumide in buffers (pH 4,5 and 6) at different temperatures (90, 100 and 120 °C)

Component	**pH 4, 90 °C, 20 min**		**pH 5, 100 °C, 100 min**		**pH 6, 120 °C, 120 min**	
	mg eq/L	% TRR	mg eq/L	% TRR	mg eq/L	% TRR
	Phenyl-label					
Pyflubumide	0.92	96	0.65	71	0.79	84
P-NH	0.04	4.4	0.18	19	0.10	11
P-aniline-isobutyryl	ND		0.09	10	0.05	5.3
P-acid	-		-		-	
Total	0.97	100	0.92	100	0.95	100

Component	pH 4, 90 °C, 20 min		pH 5, 100 °C, 100 min		pH 6, 120 °C, 120 min	
	mg eq/L	% TRR	mg eq/L	% TRR	mg eq/L	% TRR
	Pyrazole-label					
Pyflubumide	0.91	97	0.68	74	0.75	82
P-NH	0.03	3.0	0.16	18	0.11	12
P-aniline-isobutyryl	-		-		-	
P-acid	ND		0.08	8.8	0.06	6.7
Total	0.94	100	0.93	100	0.92	100

ND, not detected

-, not analysed

Apple

Apple processing studies were conducted in 2017 (Kondo K., 2018, R-32093) using apple fruits from the residue trials at three different locations in Japan with the apple variety Fuji (Takahashi Y., 2018, R-32091). In all trials apple trees were treated according to the critical GAP: once at the concentration rate of 10 g ai/hL (2000-fold dilution). The spray volume was 4500 L/ha. Apple RAC samples harvested 1 DAT were used in an apple processing study to determine the residue concentration of pyflubumide and its metabolites P-NH, P-acid and P-aniline-isobutyryl in the processed commodities of apple. The bulk apple samples were washed and then processed according to simulated commercial procedures into the apple juice (pasteurized and clarified), wet pomace, dried pomace, apple sauce (pasteurized) and dried apples. All analytical samples were extracted after homogenization or pulverization and stored at 1–10 °C until clean-up and analysis (refer to point 6.1.1 for storage stability data) by Method A.

A comparison of the residues in the RAC samples with those in each processed commodity was shown in Table 28. Pyflubumide residues including the metabolites analysed showed higher concentrations in wet and dried pomace. Residue concentrations were lower in food commodities such as juice, sauce and dried apple.

Table 28 Residues of pyflubumide in apple and its processed commodities

Location Year	Commodity	mg pyflubumide eq/kg					
		Pyflubumide	P-NH	Sum 1	P-aniline-isobutyryl	P-acid	Sum 2
Aomori 2017	Apple (RAC)	0.44	0.02	0.46	< 0.02	< 0.04	0.52
	Pasteurized juice	0.0006	< 0.0006	0.0012	< 0.0007	< 0.002	0.004
	Wet pomace	1.06	0.054	1.11	< 0.007	< 0.02	1.14
	Dry pomace	5.16	0.524	5.68	0.056	0.06	5.80
	Pasteurized sauce	< 0.005	< 0.006	< 0.011	< 0.007	< 0.02	< 0.04
	Dried apple	0.012	< 0.006	0.018	< 0.007	< 0.02	0.05
Iwate 2017	Apple (RAC)	0.52	0.03	0.55	< 0.02	< 0.04	0.61
	Pasteurized juice	< 0.0005	< 0.0006	< 0.0011	< 0.0007	< 0.002	< 0.004
	Wet pomace	1.76	0.084	1.84	< 0.007	< 0.02	1.87
	Dry pomace	8.20	0.603	8.80	0.083	0.08	8.97
	Pasteurized sauce	< 0.005	< 0.006	< 0.011	< 0.007	< 0.02	< 0.04
	Dried apple	0.010	< 0.006	0.016	< 0.007	< 0.02	0.04
Fukushima 2017	Apple (RAC)	0.43	0.02	0.45	< 0.02	< 0.04	0.50
	Pasteurized juice	0.0012	< 0.0006	0.0018	< 0.0007	< 0.002	0.005
	Wet pomace	1.58	0.071	1.65	< 0.007	< 0.02	1.68
	Dry pomace	7.84	0.559	8.40	0.062	0.06	8.52
	Pasteurized sauce	< 0.005	< 0.006	< 0.011	< 0.007	< 0.02	< 0.04

Location Year	Commodity	mg pyflubumide eq/kg					
		Pyflubumide	P-NH	Sum 1	P-aniline-isobutyryl	P-acid	Sum 2
	Dried apple	0.030	< 0.006	0.036	< 0.007	< 0.02	0.06

Sum 1: sum of pyflubumide and P-NH expressed in pyflubumide

Sum 2: sum of pyflubumide, P-NH, P-aniline-isobutyryl and P-acid, expressed in pyflubumide

Processing factors were calculated the sum of pyflubumide and P-NH (expressed in pyflubumide) in processed apple commodities as shown in Table 29.

Table 29a Processing factors for apple processed commodities (for the sum of pyflubumide and P-NH expresses in pyflubumide).

Processed commodity	Processing factor	
	Individual values	Mean or Best estimate
Pasteurized juice	< 0.002, 0.002, 0.004	0.003
Wet pomace	2.4, 3.3, 3.7	3.3
Dry pomace	12.3, 16.0, 18.7	16.0
Pasteurized sauce	< 0.02, < 0.02, < 0.02	< 0.02
Dried apple	0.029, 0.039, 0.08	0.05

Table 29b Processing factors for apple processed commodities (for pyflubumide).

Processed commodity	Processing factor	
	Individual values	Mean or Best estimate
Wet pomace	2.4, 3.4, 3.7	3.2
Dry pomace	11.7, 15.8, 18.2	15.2

Tea

Samples of dry tea leaves from eight supervised residue trials (R-32006, R-32007, R-32034 and R-32071) were used to brew tea infusions. In these trials, tea trees were treated with 5 or 10 g ai/hL (the critical GAP is 10 g ai/hL). Fresh tea leaves for processing to dry tea leaves were harvested 7 days after the application. The fresh tea leaves were processed as described in the section on supervised residue trials on tea.

For preparation of tea infusions, 540 mL of boiling water was added to 5–9 g of dry tea leaves and brewed for 5 min. The resulting extracts were filtered and analysed for pyflubumide residues. Processing factors were derived from residues in the infusions and in the dry tea leaves. As anticipated, the processing factor for brewing is very low but conversion from pyflubumide to P-NH was observed by changing ratio of these compounds.

Table 30 Residues of pyflubumide in dry tea leaves and infusion, and the processing factor from tea to infusion.

Location Year, Report No.	Commodity	mg pyflubumide eq/kg			Processing factor (brewing)
		Pyflubumide	P-NH	Sum	
Saitama	Tea dry leaves (RAC)	22.6	7.5	30.1	-
2009, R-32006	Tea infusion	0.0008	< 0.0010	0.0018	0.00006

Location Year, Report No.	Commodity	mg pyflubumide eq/kg			Processing factor (brewing)
		Pyflubumide	P-NH	Sum	
Kochi 2009, R-32006	Tea dry leaves (RAC) Tea infusion	11.8 < 0.0008	2.9 0.0023	14.7 0.0031	0.00021
Mie 2009, R-32007	Tea dry leaves (RAC) Tea infusion	0.86 < 0.0008	1.7 < 0.0010	2.5 < 0.0018	- < 0.00072
Kagoshima 2009, R-32007	Tea dry leaves (RAC) Tea infusion	23.5 0.0008	6.3 0.0071	29.8 0.0079	- 0.00027
Saitama 2009, R-32032 R-32034	Tea dry leaves (RAC) Tea infusion	1.7 < 0.0002	1.6 0.0005	3.3 0.0007	- 0.00021
Mie 2009, R-32032 R-32034	Tea dry leaves (RAC) Tea infusion	19.4 0.0004	7.8 0.0038	27.2 0.0042	- 0.00015
Saitama 2009, R-32071	Tea dry leaves (RAC) Tea infusion*	6.1 0.00013	3.0 0.0025	9.1 0.0038	- 0.0042
Kochi 2009, R-32071	Tea dry leaves (RAC) Tea infusion*	2.9 0.0007	3.6 0.0015	6.5 0.0022	- 0.0034
		Median or best estimate			0.0003

RESIDUES ON ANIMAL PRODUCTS

Livestock Feeding Studies

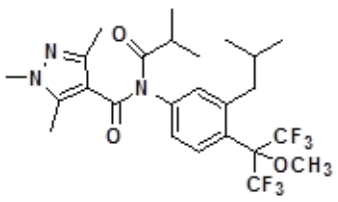
No feeding studies were received by the current Meeting.

APPRAISAL

Pyflubumide(3'-isobutyl-N-isobutyryl-1,3,5-trimethyl-4'-[2,2,2-trifluoro-1-methoxy-1-(trifluoro-methyl) ethyl]-pyrazole-4-carboxanilide) (IUPAC name) is a new pyrazole carboxamide acaricide used for control of mites. It inhibits mitochondrial electron transport system complex II (succinic dehydrogenase complex).

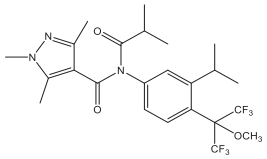
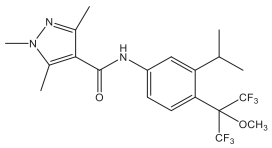
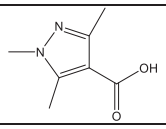
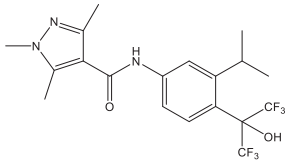
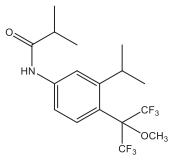
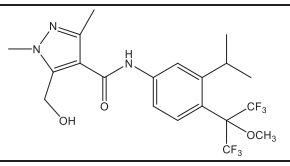
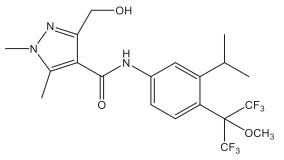
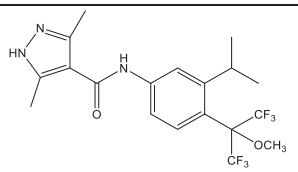
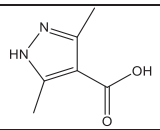
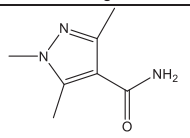
Pyflubumide was scheduled by the Forty-eighth Session of the CCPR in 2016 for toxicological and residue evaluation by the 2019 JMPR as a new compound. No specification has been established by the Joint FAO/WHO Meeting on Pesticide Specifications for pyflubumide.

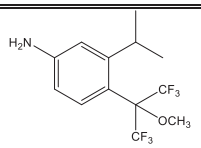
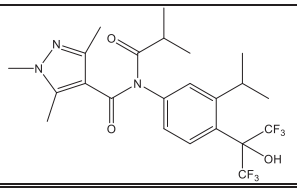
The Meeting received information on identity, physical and chemical properties, metabolism and environmental fate, residue analysis and storage stability, use pattern, supervised trials on apple and tea, and processing studies on apple and tea.



The following abbreviated names were used for the metabolites referred to in this appraisal.

Table 1 List of compounds appearing in this appraisal

Compound Name/Codes	IUPAC name	Structure
Pyflubumide/ NNI-0711	3'-isobutyl-*N*-isobutyryl-1,3,5-trimethyl-4'-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl) ethyl]pyrazole-4-carboxanilide	
P-NH/ NNI-0711-NH Pyflubumide-NH	3'-isobutyl-1,3,5-trimethyl-4´-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)ethyl]pyrazole-4-carboxanilide	
P-acid/ NNI-0711-acid Pyflubumide-acid	1,3,5-trimethylpyrazole-4-carboxylic acid	
P-NH-RfOH/ NNI-0711-NH-RfOH Pyflubumide-NH-RfOH	3'-isobutyl-1,3,5-trimethyl-4´-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]pyrazole-4-carboxanilide	
P-aniline-isobutyryl/ NNI-0711-aniline-isobutyryl Pyflubumide-aniline-isobutyryl	3'-isobutyl-4-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)- ethyl]phenyl]isobutylanilide	
P-NH-5-CH_2OH/ NNI-0711-NH-5-CH_2OH Pyflubumide-5-CH_2OH	5'-hydroxymethyl)-3'-isobutyl-1,3-dimethyl-4´-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)ethyl] pyrazole-4- carboxanilide	
P-NH-3-CH_2OH/ NNI-0711-NH-3-CH_2OH Pyflubumide-3-CH_2OH	3-(hydroxymethyl)-3'-isobutyl-1,5-dimethyl-4'-[2,2,2-trifluoro-methoxy-1-(trifuluoromethyl) ethylpyrazole-4-caroxanilide	
P-NH-1-H/ NNI-0711-NH-1-H Pyflubumide- NH-1-H	3'-isobutyl-3,5-dimethyl-4´-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)ethyl]pyrazole-4-carboxanilide	
P-acid-1-H/ NNI-0711-acid-1-H Pyflubumide- acid-1-H	3,5-dimethylpyrazole-4-carboxylic acid	
P-amide/ NNI-0711-amide Pyflubumide-amide	1,3,5-trimethylpyrazole-4-carboxamide	

Compound Name/Codes	IUPAC name	Structure
P-aniline/ NNI-0711-aniline Pyflubumide-aniline	3-isobutyl-4-[2,2,2-trifuluoro-1-methoxy-(trifuluoromethyl)ethyl] aniline	
Pyflubumide-RfOH NNI-0711-RfOH	3'-Isobutyl-N-isobutyryl-1,3,5-trimethyl-4'-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl] pyrazol-4-carboxanilide	

Based on the information on physical and chemical properties, pyflubumide is not volatile and much more soluble in organic solvents than in water with a $LogP_{ow}$ of 5.34 indicating that translocation of pyflubumide in plants is unlikely to be significant. Photolysis seemed to be the major degradation pathway of pyflubumide.

Plant metabolism

The Meeting received information on the fate of pyflubumide in apple, eggplant and spinach after one foliar spray application. For the studies, pyflubumide labelled with ^{14}C at the phenyl ring ([U-phenyl-^{14}C]-pyflubumide; abbreviated as phenyl-label hereafter) and at position 3 or 5 of the pyrazole ring ([pyrazole-3(5)-^{14}C]-pyflubumide; abbreviated as pyrazole-label hereafter) were used. In metabolism studies, total radioactive residues (TRR) are expressed in mg pyflubumide equivalents/kg.

Apple

Phenyl- or pyrazole-labelled pyflubumide was applied to apple plants, grown outdoors, as a foliar spray once at a rate of 360 or 350 g ai/ha, (concentration: ca. 10 g ai/hL). Fruit and leaf samples were collected 0–51 days after the application.

TRR after the treatment with either of the labelled pyflubumide decreased in the fruit from 0.16–0.19 mg eq/kg at 0 DAT to 0.090–0.096 mg eq/kg at 7 DAT and then to 0.058–0.068 mg eq/kg at 51 DAT. In leaves, TRR decreased from 17 mg eq/kg at 0 DAT to 12 mg eq/kg at 7 DAT and then to 5.1–5.4 mg eq/kg at 51 DAT.

Distribution of radioactivity in fruits and leaves was similar between the two ^{14}C-labelled pyflubumide treatments. Most of the radioactivity was recovered in the acetone surface wash of fruits and leaves, accounting for 77–98% TRR and 86–98% TRR, respectively at 0–7 DAT, decreasing to 54–65% TRR at 51 DAT. Acetone, acetone/water, and acetone/1 M HCl (1:1, v/v) further extracted additional radioactivity. Total extractability was 86–100% TRR throughout the study period.

The most abundant radioactive residue in the surface wash and extracts was the parent pyflubumide accounting for 88–92% TRR in fruits and leaves (16 mg/kg) at 0 DAT and decreased to 50–56% TRR at 7 DAT and then to 17–28% at 51 DAT. P-NH was the only identified metabolite. Its proportion increased from 1.2–2.7% TRR at 0 DAT to 13–18% TRR at 14–28 DAT and 12–16% TRR by 51 DAT. Its concentration peaked at 7–14 DAT around 0.013–0.018 mg eq/kg in fruits and 1.3–1.6 mg eq/kg in leaves. Beta-glucosidase did not release radioactive compounds from surface wash and acetone extract, suggesting that glucose conjugates were not present.

Total unidentified residues in washes and extracts increased over time accounting for up to 61% TRR at 51 DAT. They consisted of multiple (e.g. up to 23 peaks in surface washes) minor peaks in HPLC and each peak accounted for < 10% TRR and < 0.01 mg eq/kg.

Unextracted radioactivity increased from < 0.2% TRR at 0 DAT to a maximum of 14% TRR at 51 DAT, and the concentration in pyflubumide equivalents also increased. Treatments of the

unextracted residues from 28 and 51 DAT leaf samples with 1 or 6 M HCl or 1 M KOH at 50 °C for 4 hours released < 1% TRR but the treatment with 24% KOH released up to 5% TRR.

Eggplant

Phenyl- or pyrazole- labelled pyflubumide was applied as a foliar spray at a rate of 490 or 550 g ai/ha (ca. 20 g ai/hL) to eggplants, grown in a greenhouse, equipped with a UV-transparent ceiling.

The TRR in fruits and leaves after treatment with labelled pyflubumide decreased from 0 DAT (0.76–1.4 mg eq/kg in fruits and 55–74 mg eq/kg in leaves) to 7 DAT (0.66–0.88 mg eq/kg in fruits and 31–48 mg eq/kg in leaves). Most of the radioactivity was recovered in acetone surface wash throughout the study period in fruits and leaves: 93–99% TRR for fruits, and 86–96% TRR for leaves. Acetone and acetone/water mixture further extracted additional radioactivity. A total of 95–100% TRR in fruits and leaves were recovered in washes and extracts. A very small proportion of radioactivity remained unextracted in fruits (up to 5.0% TRR) and leaves (up to 5.2% TRR). TRR of only < 0.01–0.03 mg eq/kg was found in roots (sampled only at 14 DAT) indicating that there is little transfer from the sprayed parts of the plant to the roots.

Most of the radioactivity in the surface washes and extracts was the parent pyflubumide (90–98% TRR for fruits and 90–99% TRR for leaves) with some small amounts of radioactive metabolites/components. P-NH, also found in the apple study, P-aniline-isobutyryl, P-acid, P-NH-RfOH (only on/in leaves) and P-NH-5-CH_2OH (only on/in leaves at DAT 14) were detected. None of them exceeded 1.3% TRR in either fruits or leaves. In fruit samples they were found at a maximum of 0.01 mg eq/kg and in leaf samples up to 0.59 mg eq/kg.

Spinach

Phenyl- or pyrazole-labelled pyflubumide was applied to spinach as a single foliar spray at a rate of 550 or 570 g ai/ha (ca. 20 g ai/hL) grown in a greenhouse equipped with a UV-transparent ceiling.

A single application of either ^{14}C-labelled pyflubumide resulted in similar total radioactive residues (TRR) in leaves, decreasing over time from 12–13 mg eq/kg at 0 DAT to the lowest of 4.7–5.8 mg eq/kg at 14 DAT. TRR in roots and new leaf growth (post-application) were < 0.01–0.03 mg eq/kg indicating that there is little translocation from the sprayed parts to roots or new leaves.

The distribution of radioactivity in leaves was similar between the two ^{14}C-labelled pyflubumide treatments. Most of the radioactivity was recovered in the chloroform surface wash of leaves, accounting for 84–92% TRR (11–12 mg eq/kg) at 0–1 DAT. The radioactivity in the surface wash decreased to 83–87% TRR (5.0–6.3 mg eq/kg) at 21 DAT.

Acetone and acetone/water further extracted additional radioactivity (8.2–17% TRR at 0–1 DAT; and 13–16% TRR at 21 DAT). Total extractability was almost 100% TRR throughout the study period.

The most abundant radioactive residue in the surface wash and extracts was the parent pyflubumide accounting for almost 100% TRR (12–13 mg/kg) at 0 DAT but decreased to 83–91% (5.0–6.4 mg/kg) at 21 DAT. Only P-NH and P-acid were identified as metabolites at 14–21 DAT at a maximum of 3.2% TRR (0.19 mg eq/kg) at 21 DAT. There was one unknown metabolite detected in the extracts, which accounted for 4.1–5.0% TRR (0.28–0.31 mg eq/kg) at 21 DAT and was suspected to be a position-isomer of the parent based on its molecular weight.

Up to 6.3% TRR remained at the origin after TLC (indicating a polar fraction). The beta-glucosidase treatment decreased the radioactivity at the TLC origin and released P-acid (1.3–1.4% TRR), indicating that a fraction of the material was possibly a glucoside of P-acid.

Summary of plant metabolism

When pyflubumide was applied as a single foliar spray to apple, eggplant and spinach, metabolism of pyflubumide was qualitatively similar. Pyflubumide was the major component of the residue. Up to 6

metabolites, P-NH (apple, eggplant and spinach), P-aniline-isobutyryl (eggplant), P-acid (eggplant and spinach), P-NH-RfOH (eggplant leaf) and P-NH-5-CH_2OH (eggplant leaf) were identified. However, among them, only P-NH accounted for more than 10% TRR, with a maximum of 18% TRR (0.018 mg eq/kg) in apple and lower levels up to 3.2% TRR in eggplant and spinach (< 0.01 mg eq/kg in eggplant fruit and up to 0.19 mg eq/kg in spinach leaf).

All identified metabolites, except P-NH-5-CH_2OH found in eggplant leaf, were also reported in the rat metabolism study.

Animal metabolism

Metabolism studies on laboratory animals were reviewed in the framework of toxicological evaluation by the current JMPR. No other animal metabolism studies were provided to the Meeting.

Environmental fate

The Meeting received information on hydrolysis, photolysis and aerobic degradation in soil for pyflubumide.

Hydrolysis

Pyflubumide was hydrolysed faster at pH 9 than pH 4 or 7 in buffers. Estimated half-lives at 25 °C are 32 days at pH 4, 28 days at pH 7 and 6.6 days at pH 9. Regardless of pH, major hydrolysates which increased over time and occurred >10% AR were P-NH, P-aniline-isobutyryl and P-acid. Hydrolysis is not expected to be a significant route of degradation at environmental pH.

Photolysis in buffer and natural water

In irradiated pH 4 buffer solution, pyflubumide was rapidly decomposed with a mean half-life of 1.2 days compared to that of about 34 days in the dark controls. Therefore, irradiation was regarded to be a significant factor contributing to environmental degradation of the compound. Major degradates occurring >10% AR were P-NH, P-aniline-isobutyryl, P-acid and P-amide, which were further photolysed.

Aerobic degradation in soil

Pyflubumide degraded in a clay loam soil under laboratory conditions with a half-life of 37 days. The main degradate formed was P-NH and it reached up to 82% AR after 112 days. Consequential mineralization to carbon dioxide was confirmed and a small amount of unextracted radioactivity existed. P-NH was the only degradate found above 10% AR. Pyflubumide is not persistent in soil.

Residues in succeeding or rotational crops

No information was provided to the Meeting.

Methods of analysis

An analytical method for the determination of residues of pyflubumide in data development was provided to the current Meeting for apple and its processed commodities, as well as dry tea leaves and tea infusion.

In general, the method employs extraction by homogenization with acetone and partitioning with hexane for analysis of pyflubumide, P-NH and P-aniline-isobutyryl. The extract is cleaned up and analysed by HPLC-MS or HPLC-MS/MS. The method was validated for apple matrices and found to be suitable for data development to determine pyflubumide, P-NH, P-aniline-isobutyryl and P-acid with an LOQ of 0.01 mg/kg for apple and 0.005 mg/kg for apple processed products. The mean recoveries were within the acceptable range (71–119%). The method was also validated for tea

matrices and found to be suitable for data development with LOQs of 0.01 mg/kg in dry tea leaves and tea infusion. The mean recoveries were in the acceptable range (70–114%).

A QuEChERS method was validated and found to be suitable for multi-residue analysis with LOQs of 0.005 mg/kg for pyflubumide and P-NH in apple, grapes, wheat grain, dry tea leaves and canola seeds. The mean recoveries were in the acceptable range (74–110%).

No information on analytical methods for commodities of animal origin was submitted to the Meeting.

Stability of residues in stored analytical samples

The stability of pyflubumide and P-NH during frozen storage at -20 °C or below was investigated in homogenized apple and dry tea leaves. The control samples from supervised trials were spiked with pyflubumide or P-NH and stored under the same conditions as treated samples. These spiked samples were analysed after all the treated samples were analysed to confirm the stability of analytes. The Meeting considered that these compounds were stable in homogenized apple for at least 87 days (longer than the storage period of trial samples) and dry tea leaves for at least 107 days (longer than the storage period of trial samples) under frozen conditions.

Definition of the residue

Plant commodities

The predominant residue was parent pyflubumide: 50–92% TRR in apple fruits and leaves at 0–7 DAT, > 90% TRR in eggplant fruits during 0–14 DAT and 83–100% TRR in spinach leaves during 0–21 DAT.

Suitable analytical methods are available for plant commodities to determine pyflubumide.

The Meeting considered that pyflubumide was a suitable marker for enforcement of MRLs.

For dietary risk assessment, the Meeting noted that in the plant metabolism studies, P-NH (apple, eggplant and spinach), P-aniline-isobutyryl (eggplant), P-acid (eggplant and spinach), P-NH-5-CH_2OH (eggplant leaf) and P-NH-RfOH (eggplant leaf) were identified. Among them only P-NH accounted for >10% TRR (apple). These metabolites were also found in the rat metabolism although some of them at trace levels.

P-NH occurred at up to 11–12% AR after simulated sterilization at pH 6. It increased in proportion compared to the parent during the processing of apple.

In the supervised trials, pyflubumide and P-NH were analysed. In the apple trials P-NH was below the LOQ of 0.01 mg/kg or slightly higher (up to 0.03 mg/kg). However, in the tea trials, P-NH was sometimes found at higher levels than the parent, perhaps due to the processing of fresh leaves to dry leaves. The current Meeting noted that the ADI and ARfD covers the parent, P-NH and pyflubumide-RfOH (detected in rat but not detected in plant metabolism studies).

P-aniline-isobutyryl was below the LOQ in three apple trials in which it was analysed and <LOQ (0.007 mg eq/kg) in apple processed commodities except dry pomace (0.056–0.083 mg eq/kg). It was not analysed in the tea trials or processing studies.

P-acid was <LOQ (0.04 mg eq/kg) in apple RAC and <LOQ (0.02 mg eq/kg) in apple processed commodities except dry pomace (0.08 mg eq/kg). It was not analysed in the tea trials or processing studies.

The Meeting concluded that dietary exposure to P-aniline-isobutyryl or P-acid would be insignificant and therefore decided not to include these metabolites in the residue definition for dietary risk assessment.

The Meeting considered that in addition to pyflubumide, P-NH should be included in the residue definition for risk assessment.

Animal commodities

The Meeting did not receive information on livestock metabolism, feeding studies or analytical methods for animal commodities. There would be some dietary burden arising from the use of apple wet pomace for feed.

The Meeting considered that it is not possible to determine residue definitions for pyflubumide in animal commodities due to the lack of information.

Conclusion

Based on the above, the Meeting recommended the following residue definitions.

Definition of the residue for compliance with the MRL for plant commodities: *Pyflubumide.*

Definition of the residue for for dietary risk assessment for plant commodities: *Sum of pyflubumide and 3'-isobutyl-1,3,5-trimethyl-4′-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)ethyl]pyrazole-4-carboxanilide (P-NH), expressed as pyflubumide*.

Results of supervised residue trials on crops

The Meeting received supervised trial data for pyflubumide on apple and tea.

Apple

Critical GAP in Japan for apple allows one application at a concentration of 10 g ai/hL and a PHI of 1 day.

Ten supervised trials were conducted on apple in Japan. Pyflubumide was applied once as foliar spray at a spray concentration of 10 g ai/hL (8 trials) or 20 g ai/hL (2 trials).

Pyflubumide from trials matching the critical GAP in Japan were in rank order (n = 8): 0.13, 0.23, 0.23, 0.34, 0.44, 0.45, 0.46 and 0.52 mg/kg.

Total residues (sum of pyflubumide and P-NH expressed in pyflubumide) from trials matching the critical GAP in Japan were in rank order (n = 8): 0.15, 0.25, 0.25, 0.36, 0.46, 0.47, 0.48 and 0.55 mg/kg.

The Meeting estimated a maximum residue level of 1 mg/kg, STMR of 0.41 mg/kg and HR of 0.55 mg/kg for apple.

The Meeting noted that the calculated IESTI for raw apples were up to 160% of the ARfD for the general population and up to 390% for children. However, no alternative GAP is available.

Tea

Critical GAP in Japan for tea allows one application at a concentration of 10 g ai/hL and PHI of 7 days.

Eight independent supervised trials were conducted on tea in Japan. Pyflubumide was applied once as a foliar spray at a concentration of 10 g ai/hL (6 trials) or 5 g ai/hL (2 trials).

Pyflubumide in dried green tea leaves from trials matching the critical GAP in Japan were in rank order (n = 6): 1.7, 2.9, 6.1, 12, 19 and 23 mg/kg. In two other trials where spray concentrations were 5 g ai/ha with the same water volume, unscaled pyflubumide residues were: 0.85 and 25 mg/kg. Using a scaling factor of 2, scaled residues were: 1.7 and 50 mg/kg.

Combined pyflubumide residues were in rank order (n = 8): 1.7, 1.7, 2.9, 6.1, 12, 19, 23 and 50 mg/kg.

Total residues (sum of pyflubumide and P-NH expressed in pyflubumide) from trials matching the critical GAP in Japan were in rank order (n = 6): 3.3, 6.5, 9.1, 17, 27 and 34 mg/kg. In other trials at a concentration two times higher, unscaled total residues were: 3.1 and 34 mg/kg. Using a scaling factor of 2, scaled residues were: 6.2 and 68 mg/kg.

Combined total residues were in rank order (n = 8): 3.3, 6.2, 6.5, 9.1, 17, 27, 34 and 68 mg/kg.

The Meeting estimated a maximum residue level of 80 mg/kg and STMR of 13 mg/kg for tea, green, black (black, fermented and dried).

The calculated IESTI for tea leaves were up to 230% of the ARfD for the general population and up to 150% for children. However, the calculated IESTI for tea infusion were 2% of the ARfD for general population (no value for children). The Meeting noted that as the $LogP_{ow}$ of pyflubumide is 5.34 and P-NH has a similar structure, it is unlikely that tea infusion would contain pyflubumide or P-NH at concentrations higher than the detection limit.

Fate of residues during processing

High temperature hydrolysis

The hydrolysis of phenyl-labelled and pyrazole-labelled pyflubumide was studied in a sterile buffered aqueous solution under conditions simulating pasteurization, baking/brewing/boiling, and sterilization.

Pyflubumide was stable under the condition representing pasteurization (pH 4, 90 °C, 20 min) with 96–97% AR recovered at the end of incubation. Under baking/brewing/boiling (pH 5, 100 °C, 60 min) and sterilization (pH 6, 120 °C, 20 min) conditions, 71–74% and 82–84% AR was recovered as parent at the end of incubation, respectively. Degradation products identified were: pasteurization, P-NH (3.0–4.4% AR); baking/brewing/boiling, P-NH (18–19% AR), P-aniline-isobutyryl (10% AR) and P-acid (8.8% AR); sterilization, P-NH (11–12% AR), P-aniline-isobutyryl (5.3% AR) and P-acid (6.7% AR). No other degradation products were detected.

Processing

The Meeting received information on processing of apple to pasteurized juice, wet pomace, dry pomace, pasteurized sauce and dried apple; and dry tea leaves to tea infusion. Processing factors of apple processed products and tea leaves to tea infusion are summarized below together with STMR-P values.

Table 2 Processing factors for apple processed commodities and tea infusion for dietary risk assessment (sum of pyflubumide and P-NH expressed as pyflubumide)

Processed commodity	Individual processing factor	Mean or Best estimate	STMR/STMR-P	HR/HR-P
Apple			0.41	0.55
Pasteurized juice	< 0.002, 0.002, 0.004	0.003[a]	0.001	-
Pasteurized sauce	< 0.02, < 0.02, < 0.02	< 0.02	0.008	-
Dried apple	0.029, 0.039, 0.08	0.05	0.02	0.028
Tea leaves, dry			13	-
Tea infusion	0.00006, 0.00015, 0.00021, 0.00021, 0.00027, 0.00034, 0.00042, < 0.00072	0.0003	0.004	-

[a] Mean of two finite processing factors

Table 3 Processing factors for apple processed commodities for animal dietary burden calculation (pyflubumide only)

Processed commodity	Individual processing factor	Mean or Best estimate	Median residue
Apple			0.39
Wet pomace	2.4, 3.4, 3.7	3.2	1.2
Dry pomace	11.7, 15.8, 18.2	15.2	5.9

Using the best estimates of processing factors and the STMR values for apple and dry tea leaves, the STMR-P values were calculated for processed commodities of apple and tea infusion.

The median residue for apple wet pomace was calculated for animal dietary burden calculation.

Residues in Animal Products

No feeding study was conducted on cattle or laying hens.

As no livestock metabolism studies or analytical method for foods of animal origin was available, the Meeting did not establish residue definitions for animal commodities. Therefore, the Meeting did not calculate animal dietary burden.

The Meeting concluded it was not possible to estimate maximum residue levels for foods of animal origin.

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 and IESTI assessment.

Definition of the residue for compliance with the MRL for plant commodities: Pyflubumide

Definition of the residue for dietary risk assessment for plant commodities : Sum of pyflubumide and 3'-isobutyl-1,3,5-trimethyl-4´-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)ethyl]pyrazole-4-carboxanilide, expressed as pyflubumide

Table 4 Residue levels suitable for estimating maximum residue limits and for IEDI and IESTI assessment

CCN	Commodity	Recommended maximum residue level mg/kg		STMR or STMR-P mg/kg	HR mg/kg
		New	Previous		
FP 0226	Apple [a]	1[a]	-	0.41	0.55
DT 1114	Tea, Green, Black (black, fermented and dried) [a]	80 [a]	-	13	-

[a] On the basis of the information provided to the JMPR it was concluded that the estimated acute dietary exposure to residues of pyflubumide for the consumption of apple and tea may present a public health concern

Table 5 Values used in calculating dietary exposure

CCN	Commodity	Recommended maximum residue level mg/kg		STMR-P mg/kg	HR/HR-P mg/kg
		New	Previous		
JF 0226	Apple juice	-	-	0.001	-
	Apple sauce	-	-	0.008	-

DF 0226	Apples, dried	-	-	0.02	0.028
	Tea infusion			0.004	-

Table 6 Values used in calculating animal dietary burdens

Commodity		Median residue mg/kg
CCN	Name	
	Apple pomace, wet	1.2
AB 0226	Apple pomace, dry	5.9

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The current Meeting established an ADI of 0–0.007 mg/kg bw.

The International Estimated Dietary Intakes (IEDIs) of pyflubumide were calculated for the 17 GEMS/Food Consumption Cluster Diets using the STMR or STMR-P values estimated by the current Meeting. The results are shown in Annex 3 of the 2019 JMPR Report.

The calculated IEDIs were 3–20% of the maximum ADI (0.007 mg/kg bw). The Meeting concluded that the long-term exposure to residues of pyflubumide resulting from the uses considered by the current JMPR is unlikely to present a public health concern.

Acute dietary exposure

The current Meeting established an ARfD of 0.008 mg/kg bw.

The International Estimated Short-Term Intakes (IESTIs) of pyflubumide were calculated for commodities using the HRs and STMRs/STMR-Ps estimated by the current Meeting. The results are shown in Annex 4 of the 2019 JMPR Report.

The calculated IESTIs were 1–230% of the ARfD for the general population and 1–390% of the ARfD for children.

For apple (raw), the IESTI represents 160% of the ARfD for the general population and 390% for children. For tea (dried leaf), the IESTI represents 230% of the ARfD for the general population and 150% for children. No alternative GAPs for apple or tea were available. On the basis of the information provided to the JMPR, the Meeting concluded that the acute dietary exposure to pyflubumide from the consumption of apple and tea may present a public health concern.

The Meeting also concluded that the acute dietary exposure to pyflubumide from the consumption of apple processed commodities and tea infusion is unlikely to present a public health concern.

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A-32019	Watanabe Y.	2018	Independent laboratory validation of methodology for the determination of residues of Pyflubumide and its metabolite NNI-0711-NH in apple, 85763

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A-32021	Feten E.and Van Middlesworth B.	2019	Development and Validation of a Method for the Determination of Residues of Pyflubumide and NNI-0711-NH in Crop Matrices by LC-MS/MS
A-32022	Whiting S.	2019	Independent Laboratory Validation of "Development and Validation of a Method for the Determination of Residues of Pyflubumide and NNI-0711-NH in Crop Matrices by LC-MS/MS"
E-32001	Masaki T.	2012	Hydrolytic fate of NNI-0711
E-32002	Masaki, T.	2012, Amendment in 2012	Photodegradation of NNI-0711 in buffer solution
E-32003	Masaki T.	2011	Photodegradation of NNI-0711 in natural water
E-32004	Masaki, T.	2011, Amendment in 2012	Fate study of NNI-0711 in aerobic soil
PC-32005	Hori K.	2009	Measurement of density for NNI-0711 (pycnometer method)
PC-32006	Hori K.	2009	Measurement of solubility in organic solvents for NNI-0711 by flask method
PC-32008	Brekelmans Ir. M.J.C.	2011	Determination of physico-chemical properties of NNI-0711
PC-32009	Masaki T.	2010	n-Octanol / Water Partition Coefficient of NNI-0711
PC-32010	Masaki T.	2010	Solubility of NNI-0711 in distilled water
PC-32011	Ihara T.	2010	Ultraviolet/visible absorption spectrum of NNI-0711
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PC-32015	Masaki T.	2012	n-Octanol / Water Partition Coefficient of NNI-0711-NH
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R-320034	Imura M.	2010	Determination of residues of NNI-0711 and NNI-0711-NH in Tea Infusion of Processed Tea Leaves Treated with NNI-0712B Flowable (admixture of NNI-0711 & Fenpyroximate)
R-32004	Odanaka Y.	2009	Determination of residues of Pyflubumide in the Processed Tea Leaves Treated with 20.0% Flowable of Pyflubumide

Code	Author(s)	Year	Title, Institution, Report reference
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