AMITROLE (079)

EXPLANATION

Amitrole was first considered by the JMPR in 1974. It was re-evaluated in 1993 within the CCPR Periodic Review Programme, and a temporary ADI was allocated. A full ADI was allocated in 1997. No MRLs have been established, but it is stated that uses of amitrole should be restricted to those where residues in food would not be expected to occur. The 1993 Meeting recommended a further note that "A realistic limit of determination for the general monitoring of amitrole would be 0.05 mg/kg". This evaluation is within the CCPR Periodic Review Programme. New data on metabolism in rats and environmental fate, in addition to residue trials on apples, pears, peaches and grapes were reported by the manufacturer. The governments of The Netherlands and Poland provided information on analytical methods and national MRLs.

IDENTITY

ISO common name: amitrole

Chemical names:

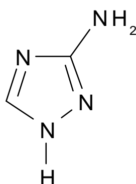
IUPAC: 1*H*-1,2,4-triazol-3-ylamine
CA: 1*H*-1,2,4-triazol-3-amine

CAS Registry. No.: 61-82-5

CIPAC No.: 90

Synonyms: aminotriazole (common name in France and Spain)

Structural formula



Molecular formula: $C_2H_4N_4$

Molecular weight: 84.08

Physical and chemical properties

Pure active ingredient

Appearance: white to yellowish crystalline powder
Vapour pressure: 3.3×10^{-5} Pa at 20°C.

Melting point: 155°C

Octanol/water partition coefficient: log P_{ow} = -0.93 at pH 4.5, -0.77 at pH 7.1, -0.80 at pH 9.9

Solubility: in water at 20°C: >1384 g/l at pH 4.0 (amitrole chlorohydrate)

264 g/l at pH 7.0
261 g/l at pH 10.0

Specific gravity: 1.54 g/cm^3 at 20°C

Hydrolysis: indefinitely stable in sterile water.

Photolysis: photolytically stable.

Dissociation constant: pK_a = 4.0; other values (for pH <2 and pH >9) are still under evaluation.

Thermal stability: stable up to 269°C

Technical material

Minimum purity: 90%

Main impurities: 7 impurities (6 identified), typically amounting 0.2 to 1.9%. The most abundant impurities are formyl-amitrole (maximum 1.9%) and 3,4-diamino-amitrole (maximum 1.1%). All other impurities are <1%.

Solubility in organic solvents at 20°C:

acetone:	2.9-3.3 g/l
1,2-dichloroethane:	<0.1 g/l
ethyl acetate:	1 g/l
n-heptane:	<<0.1 g/l
p-xylene:	<<0.1 g/l
methanol:	133-160 g/l

Melting range: 150 - 153°C

Stability: stable under storage conditions (≥2 years)

Formulations

As of February 1990, two types of product were commercialized: soluble concentrates (SL) containing 200 g/l to 500 g/l amitrole and water-soluble powders (SP) containing 50% to 90% amitrole. A water-soluble granule (SG) containing 86% amitrole is presently under registration in many countries world-wide.

METABOLISM AND ENVIRONMENTAL FATE

Animal metabolism

Four studies of the metabolism of [5-^{14}C]amitrole in rats were carried out. In all cases, amitrole was the main elimination product in the urine, and its metabolism dose-related. Up to three metabolites were detected. The proportion of metabolites in the urine after 24 hours was higher in rats dosed at low than at high levels (Fang *et al*. 1966). Within 23 hours of dosing the amount and proportion of amitrole excreted in the urine had declined markedly (Table 1).

Table 1. Radioactivity in the urine of rats after oral doses of [5-^{14}C]amitrole.

Time after dose (hours)	Amitrole		Metabolite 1		Metabolite 2	
	cpm	% of TRR	cpm	% of TRR	cpm	% of TRR
0 - 3	183 800	95	9 680	5	0	0
3 - 7	487 430	79	129 570	21	0	0
7-11	220 460	73	75 500	25	6 040	2
11-23	86 400	60	53 280	37	4 320	3

Amitrole is quickly metabolised in the liver. Fang *et al*. (1964) showed that the proportion of amitrole declined to 50% of the radioactivity in the liver by 3 hours after dosing (Table 2).

Table 2. Distribution of radioactivity extracted with alcohol from the liver of rats killed at different times after dosing with [^{14}C]amitrole.

Time (hours)	Amitrole, %	Metabolites, %
0.5	100.0	0
1	95.4	4.6
2	80.4	19.6
3	46.2	53.0
4	36.4	63.6
6	45.5	54.5
12	42.3	57.7
18	34.5	65.5
24	0	100.0

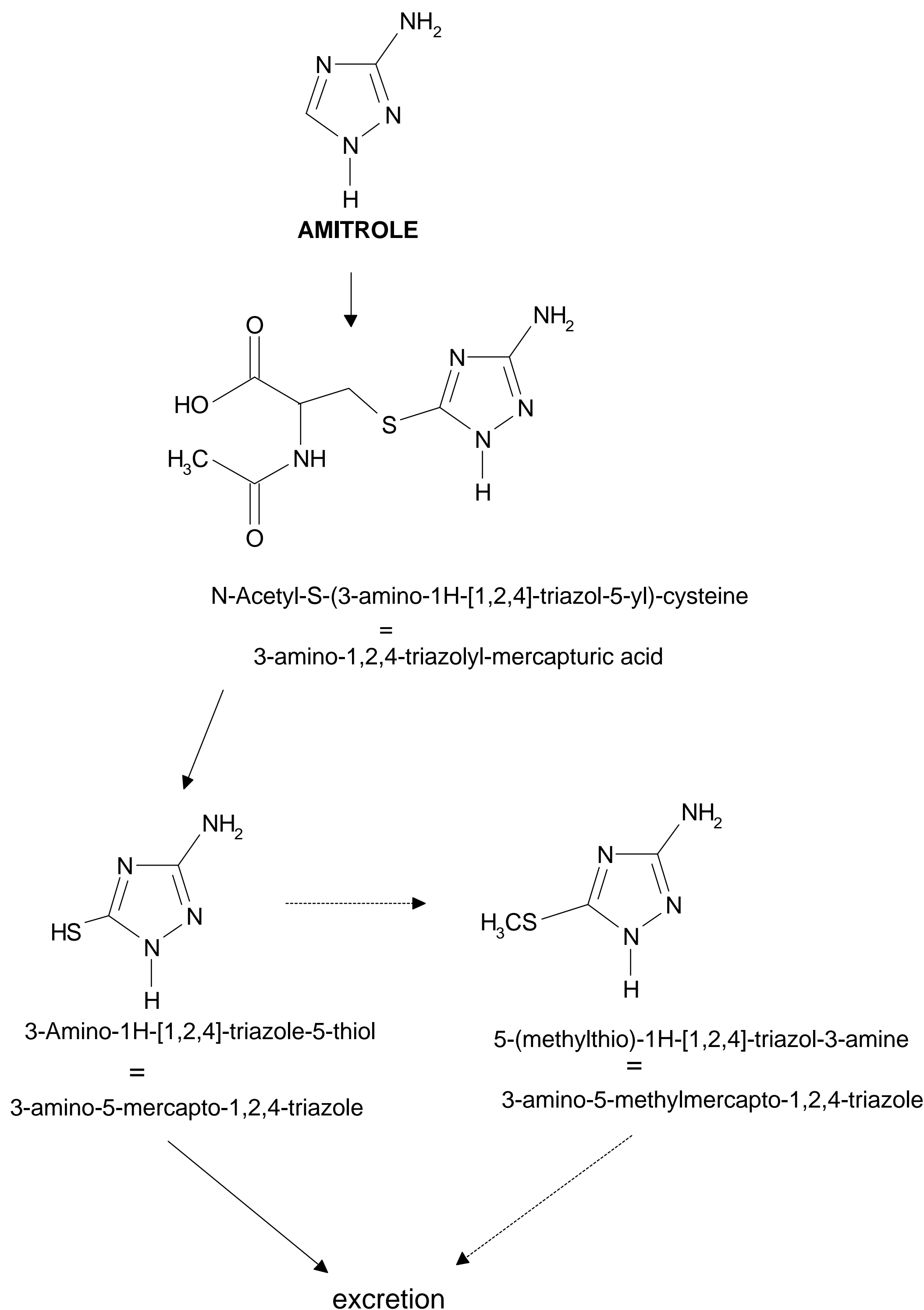
In an experiment with the ^{14}C and ^{3}H-labelled compounds, amitrole was metabolised in rats mainly at the 5-position (Table 3). The average ratio of ^{3}H to ^{14}C for the active ingredient fraction isolated from the urine was 8.6, and for the metabolite fraction 1.1 (Fang *et al.*, 1966).

Table 3. Ratio of ^{3}H to ^{14}C in amitrole and metabolite fractions from the urine of rats treated with ^{3}H- and 5-^{14}C-labelled amitrole (ratio 11/1).

Dose	Ratio ^{3}H/^{14}C											
	Rat n° 68 F		Rat n° 69 M		Rat n° 70 F		Rat n° 71 M		Rat n° 72 F		n°73 M	
	Amitr	Metab	Amitr	Metab	Amitr	Metab	Amitr	Metab	Amitr	Metab	Amitr	Metab
1	9.0	1.7	7.7	0.6	9.1	1.7	8.7	1.2	9.4	1.3	9.1	0.6
2	9.1	2.2	7.3	0.7	8.4	0.9	8.2	1.0	-	-	-	-
3	8.7	1.3	7.8	0.5	8.1	0.6	7.3	0.6	-	-	-	-
4	9.2	1.7	8.6	0.9	9.1	0.9	8.5	0.8	-	-	-	-
5	9.0	1.8	9.7	0.9	-	-	-	-	-	-	-	-
^{3}H/^{14}C amitrole for male rats: 8.30 ± 0.7												
^{3}H/^{14}C amitrole for female rats: 8.90 ± 0.3												
^{3}H/^{14}C metabolites for male rats: 0.78 ± 0.20												
^{3}H/^{14}C metabolites for female rats: 1.42 ± 0.46												

The proposed degradation pathway is shown in Figure 1.

Figure 1. Metabolism of amitrole in rats.



Grunow *et al.* (1975) confirmed that amitrole is metabolised in rats by substitution at the 5-carbon atom. The urine of rats 24 hours after a 50 mg/kg bw dose contained unchanged amitrole and small amounts of 3-amino-5-methylthio-1,2,4-triazole, 3-amino-5-mercapto-1,2,4-triazole and 3-amino-1,2,4-triazolyl-5-mercapturic acid, identified by comparative paper and/or thin-layer chromatography in several mobile solvent systems as well as comparative IR and mass spectrometry. The alanine conjugate postulated by Franco and Municio (1975) could not be detected by Grunow *et al.*

In another more recent study (Anderson and Brauner, 1995) [5-^{14}C]amitrole was administered orally or intravenously to four groups of 5 rats to study the dependence of kinetic parameters on the route of administration (oral or i.v.), dose (1 or 500 mg/kg), pre-treatment and sex.

Absorption from the gastrointestinal tract started immediately after administration and the peak plasma level was reached after 40 to 60 minutes in all groups. The test compound was distributed extensively into peripheral compartments. Under steady state conditions the radioactivity permeated readily into the tissues and, with a very low mean residence time, back into the plasma before elimination. In rats killed after short times increased concentrations of radioactivity (whole-body autoradiography) were recognisable in all organs and tissues except bone marrow, brain, eye and brown fat. Owing to the fast excretion, radioactivity in all organs and tissues was depleted quickly. In rats killed 48 hours after administration of the compound, radioactivity was detected mainly in the liver and to a small extent in the kidney cortex and nasal mucosa. Recoveries of radioactivity were on average higher than 93.8% of the dose.

Biotransformation to volatile metabolites including carbon dioxide was negligible (0.1% of the administered dose). More than 97% of the recovered radioactivity was excreted within 48 or 72 hours after oral administration. The major elimination route was renal, with the high-dose animals excreting 97 to 98% and the low-dose 91 to 95% in the urine, irrespective of the administration route. Repeated oral doses resulted in urinary excretion in the same range. Faecal elimination accounted for 1.6 to 6.3%. The residues in the rat bodies excluding the gastrointestinal tract were significantly lower after a single high dose than after single or repeated low doses. Radioactivity after a single intravenous dose was slightly lower than after a single oral dose and slightly lower in female than in male rats after single and repeated oral doses. The half-life of elimination was shorter after intravenous administration than after oral dosing.

Analysis of the urine by chromatography established that a very high percentage of amitrole passed through unchanged, accounting for more than 86% of the identified radioactivity and for two thirds or more of the administered dose. Small amounts of 7 different metabolites were detected, one of which was identified by mass spectrometry. Two other biotransformation products were identified by chromatography with the authentic reference compounds on thin-layer plates and by HPLC. The proportion of identified compounds was high and independent of the route of administration or sex, ranging from about 76 to 93% of the recovered radioactivity.

In a further study (Iatropoulos *et al.*, 1996), amitrole was administered in the NIH-07 diet *ad libitum* to F344 rats and C57BL/6N mice at concentrations of 0, 1, 10, 100 or 1000 mg/kg in the diet. Animals were killed after 4 or 21 days of exposure. Three days before being killed at 21 days, four of the ten animals in each group were given bromodeoxyuridine (BrdU) which was incorporated into proliferating cells. In both rats and mice the blood levels of radioactivity were proportional to the dose but lower in mice, indicating probably more effective liver enzyme induction and systemic elimination. After 21 days serum levels were identical to those after 4 days in rats, but lower in mice. In rats amitrole at 100 and 1000 ppm caused significant time- and dose-related decreases in body weight. Liver weights were decreased, although hepatocellular BrdU labelling indices were increased, reaching a maximum of a 3-fold increase compared to controls. Hepatic glutathione peroxidase activity was inhibited at 1000 ppm. Thyroid follicular cell hyperplasia was induced at 10 ppm and above. In mice amitrole at the same dose levels did not cause body weight changes. Liver weights were increased by day 21, increases in hepatocellular BrdU labelling indices were evident, and hepatic glutathione peroxidase activity was inhibited at 100 and 1000 ppm. At those levels, thyroid hyperplasia was induced, reaching a maximum of 8-fold.

Plant metabolism

The metabolism of amitrole was studied after application of [3,5-^{14}C]amitrole (8 kg/ha) to the soil under apple trees under outdoor conditions and in tubs, to excised apple tree sprouts, and to cell suspension cultures (Schneider *et al*., 1991). Mature fruit from outdoor experiments contained a maximum of 0.05 mg/kg total radioactive residues of which about 75% was soluble and 25% bound to insoluble material, but amitrole itself was not detected. The main metabolite ([0.012 mg/kg, 22-24% of the ^{14}C) was 2-amino-3-(1,2,4-triazol-l-yl)propionic acid (triazolylalanine) which occurred in the free form and as conjugates. More than 50% of the radioactivity was incorporated into natural plant constituents, some of which were insoluble and characterized after treatment with cellulase/pectinase. In the tub experiments, one apple tree absorbed 1.1% of the radioactivity applied to the soil, 0.07% was found in the mature fruit and about 42% remained in the soil after 5 months.

In contrast to the results of outdoor experiments, the main metabolite in model experiments with excised apple sprouts and most cell suspension cultures was 3-(3-amino-1,2,4-triazol-l-yl)-2-aminopropionic acid (aminotriazolylalanine), but only small amounts were present. In cell suspension cultures at high concentrations of amitrole 3,5-dihydroxy-1,2,4-triazole was the main product. The distribution of radioactivity and the proposed metabolic pathways are shown in Tables 4 and 5 and Figure 2.

Table 4. Nature of residues in harvested apples following application of [3,5-^{14}C]amitrole.

Sample	1985[1]			1986[2]		
	Radioactivity		mg/kg, as amitrole	Radioactivity		Residue, mg/kg
	Bq/g FW[3]	%		Bq/g FW[3]	%	
Total radioactivity	16.4	100.0	0.0456	19.4	100.0	0.0322
Soluble radioactivity	11.2	68.3	0.0311	14.7	75.8	0.0244
Incorporated radioactivity	6.0	36.6	0.0167	7.8	40.2	0.0130
Basic metabolites	0.7	4.3	0.0019	1.5	7.8	0.0025
Triazolylalanine and conjugate	3.6	21.9	0.0100	4.7	24.2	0.0078
Not determined	0.9	5.5	0.0025	0.7	3.6	0.0012
Radioactivity in cell wall components	5.2	31.7	0.0145	4.7	24.2	0.0078
Re-assimilated radioactivity	3.4	20.7	0.0095	3.0	15.5	0.0050
Bound metabolites	1.5	9.2	0.0042	1.6	8.2	0.0027
Not determined	0.3	1.8	0.0008	0.1	0.5	0.0002

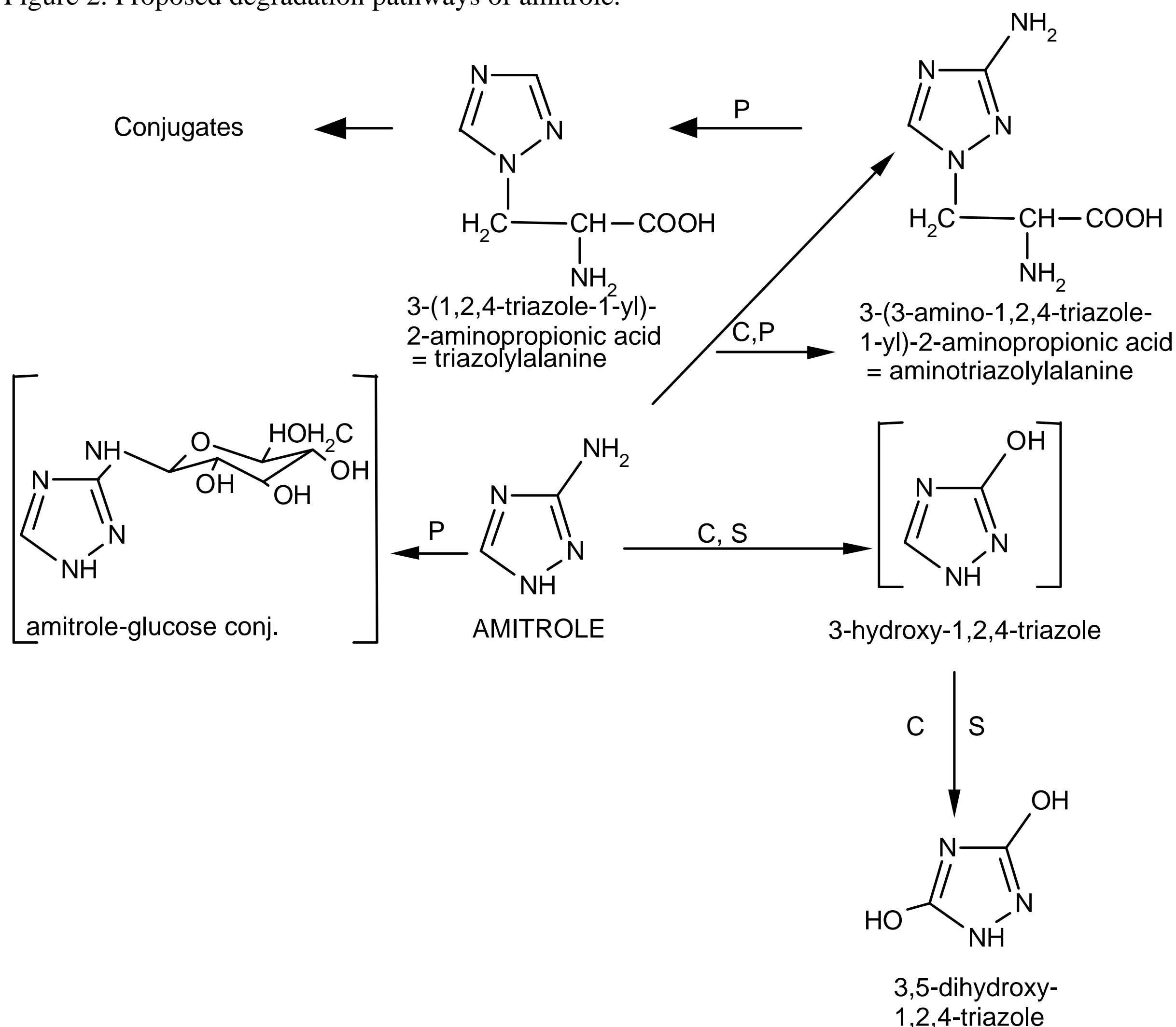
[1]Specific radioactivity 0.359 MBq/mg
[2]Specific radioactivity 0.602 MBq/mg
[3]FW: Fresh weight of harvested fruit

Table 5. Radioactivity balance at harvest (14/10/1986) following application of 400 mg [3,[5-^{14}C]amitrole (61.3 MBq/mmol) in 2 doses of 200 mg on 14/05 and 9/07/1986 to one apple tree of cultivar "Gelber Köstlicher" in a tub.

Applied radioactivity	292.000 (kBq)	100.00 (%)
Radioactivity in tree	3 240.2	1.11
Fruit	204.7	0.07
Leaves	83.9	0.03
Trunk and roots	2 951.6	1.01
One year old branches	405.4	0.14
Two year old branches	51.5	0.02
Branches older than two year	210.7	0.07
Trunk	1 312.0	0.45

Applied radioactivity	292.000 (kBq)	100.00 (%)
Rootstock	503.0	0.17
Roots	469.0	0.16
Radioactivity in soil	122 768.3	42.04
Radioactivity found(soil + tree)	126 008.5	43.15
Radioactivity lost (atmosphere)	165 991.5	56.85

Figure 2. Proposed degradation pathways of amitrole.



P = Plant, S = Soil, C = Cell culture

Environmental fate in soil

Amitrole has been extensively investigated with respect to its route and rate of degradation, adsorption/desorption, and leaching potential.

Routes of degradation

Degradation was studied in the laboratory in German standard soil 2.2 and an English loam. Amitrole was labelled with ^{14}C in the 3 and 5 positions, and the soil was kept in the dark. Under aerobic conditions after 28 days the production of $^{14}CO_2$ in the German standard soil 2.2 was 70% of the applied radioactivity, and in the English loam soil 40-50%, but in strictly anaerobic soil no

volatile radioactivity was produced. In the aerobic soil most of the involatile radioactivity was unextractable and the main extractable radioactive compound was unchanged amitrole. No intermediates in the degradation to $^{14}CO_2$, including urea and cyanamide observed in other experiments, were detected in the soil extracts. In anaerobic soil the total unbound radioactivity decreased to 60% after 28 days and to 25% after one year.

When soil under aerobic conditions was sterilized there was no significant loss of radioactivity, indicating that degradation to CO_2 is strongly influenced by micro-organisms and oxygen. Similar results were obtained with [3-^{14}C]- and [5-^{14}C]amitrole (Hawkins *et al.,* 1982).

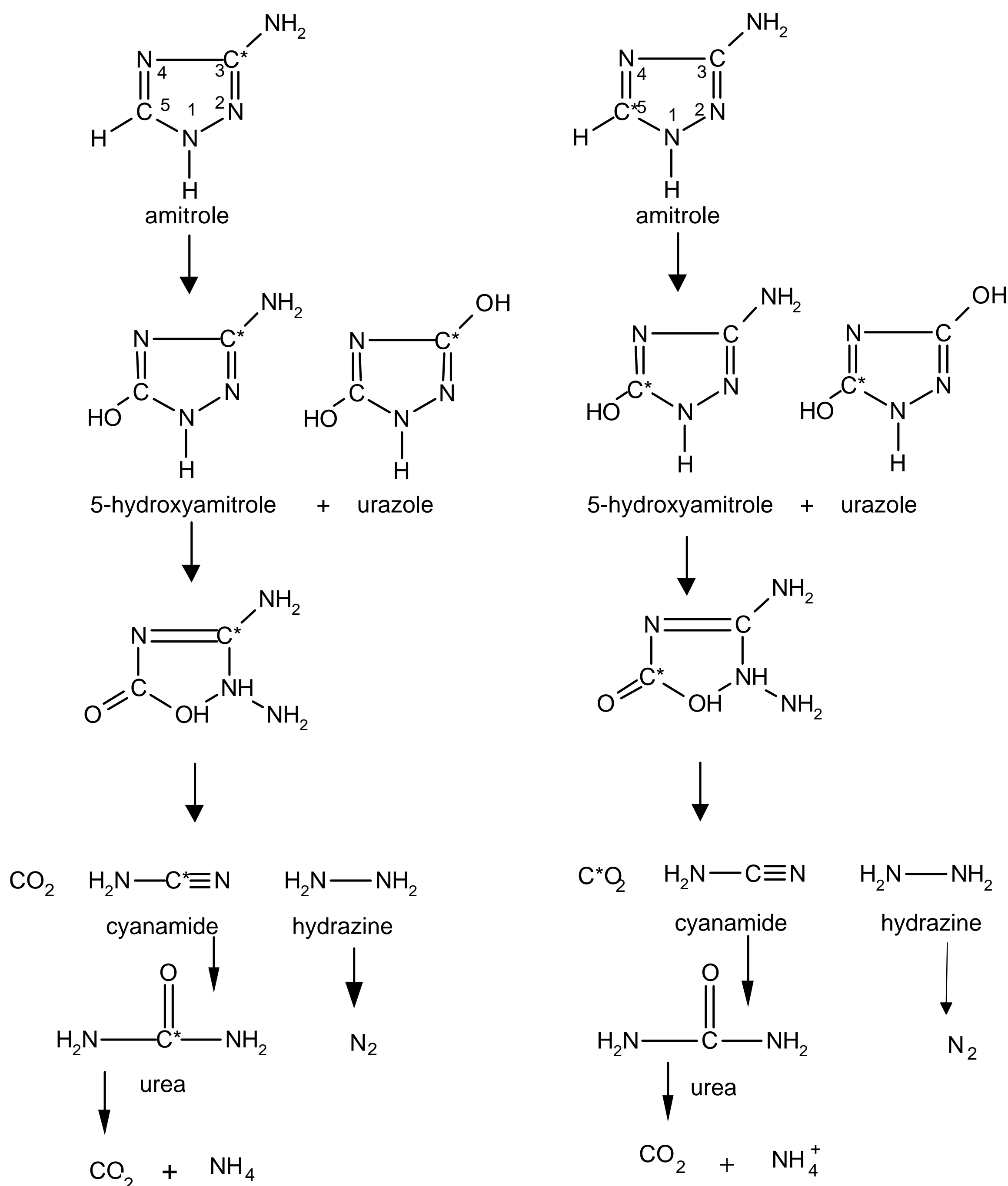
An additional laboratory experiment (Scholz, *et al*., 1988) was carried out with amitrole labelled at the 3 or 5 positions with the aim of identifying the intermediate compounds involved. A clayish silt soil was treated with 0.1 and 1.0 mg [^{14}C]amitrole/100 g soil and incubated from 1 to 20 days. Amitrole (both labelled forms) was rapidly degraded to CO_2. The residue in the soil extract consisted mainly of the parent compound, with <2.5% as metabolites. The primary metabolite was 5-hydroxy-amitrole but it was degraded very rapidly. Cyanamide and urea were also found as degradation products. Urazole (3,5-dihydroxy-1,2,4-triazole) presumably represents a secondary pathway in the metabolism. In the proposed degradation scheme, the ring is opened between positions 1 and 5 of 5-hydroxy-amitrole, CO_2 is eliminated at position 5 and hydrazine at positions 1 and 2, leaving cyanamide which is hydrolysed *via* urea to CO_2 and ammonia (Figure 3).

This degradation to simple organic compounds was confirmed in a more recent study (Hall, 1994) which showed CO_2 as the most abundant product, with urazole and aminoguanidine putatively identified at levels of ≤2%. Finally the presence of cyanamid at a very low concentration (≤0.02 mg/kg maximum at day 1) was confirmed in a recent field study (Biever, 1995).

The photodegradation of [3,5-^{14}C]amitrole has been studied by means of TLC plates of California sandy loam either developed immediately, stored in the dark, or exposed to natural sunlight or Chroma 50 lamps. Amitrole was degraded in dry soil in the dark with a half-life of approximately 330 hours. When exposed to light the half-life was approximately 10 to 20 hours of equivalent solar exposure. The products formed by exposure did not migrate from the soil in methanol or methanol/ethyl acetate/ammonium hydroxide, but when water/ammonium hydroxide was used as the solvent the products of photodegradation were shown to comprise at least two components. The smaller fraction was bound to the soil but the larger fraction migrated in the water. Although not clearly identified, these products were presumed to be cyanamide and urea (Huhtanen, 1985a).

In an investigation of the photodegradation of [^{14}C]amitrole on soil under artificial sunlight the parent concentrations without irradiation varied from 86.6% to 91.1% without any cleasr decline pattern. Under irradiated conditions however the parent concentrations decreased from 91.1% to 66.4% by 30 days. There was only one degradation product under both conditions, identified as 1,2,4-triazole. This reached a maximum concentration of 4.1% by day 21 without irradiation and 9.9% by day 30 when irradiated. The calculated photolytic half-life of amitrole was 73 days, each day consisting of 12 hours irradiation and 12 hours darkness (Das, 1990).

Figure 3. Degradation scheme for [3-^{14}C]amitrole and [5-^{14}C]amitrole in soil.



Rate of degradation

Laboratory studies. Technical amitrole was incubated in 3 German standard soils at 22°C for 17 days after treatment at 0.2 mg ai/kg soil. Amitrole was determined after extraction with acetone/water and purification on cation exchange and aluminium oxide columns by HPLC with ion pairing and electrochemical detection (limit of determination 0.02 mg/kg). The calculated half-lives were 2.4, 2.4 and 4.6 days (Jarczyk, 1982 b-d).

More recently (Hall, 1994,1995) the rate of degradation of amitrole was studied on a very light soil, a loamy sand originating from the USA. The study was conducted according to US-EPA

and Canadian requirements, at 7°C and 22°C. A half-life of 87 days was calculated at 7°C, decreasing to 26 days at 22°C.

Field studies. A terrestrial dissipation study (Biever, 1995) was conducted during 1993 in Hillsboro, Oregon and Moses Lake, Washington (close to the Canadian border) to determine the persistence of amitrole and one of its degradation products, cyanamide, in soil. At both locations the surface soil was a loam with 1.3 to 1.8% organic matter. Amitrole, formulated as Amizol®, was applied to 0.1 ha plots of bare soil at an average rate of 8.7 kg ai/ha, 97% of the maximum labelled application rate for non-crop use in the USA. The amitrole concentrations in the soil were highest on day 0 at both sites, 1.77 mg/kg at Oregon and 2.50 mg/kg at Washington. The decrease after 7 days was rapid with concentrations at the limit of quantification (LOQ) by 124 days at Oregon and 131 days at Washington. Mean monthly temperatures ranged from 13°C to 19°C at Oregon and 10°C to 21°C at Washington. The theoretical half-life of amitrole in soil was 17 days (r = 0.98) in Oregon and 21.0 days (r = 0.96) in Washington.

Adsorption and desorption

One g portions of soil were equilibrated with 10 ml of solutions containing 0.51 to 10.1 mg/l of [3,5-^{14}C]amitrole for 2 hours at 25°C, the samples were centrifuged and the supernatants analysed. A similar procedure was followed for desorption. The results are summarized in Table 6. Amitrole was adsorbed to some extent by all the soils studied. Adsorption followed a Freundlich isotherm. Freundlich constant (K_d) values ranged from 0.685 to 3.79. Desorption of adsorbed amitrole ranged from 0.7 to 52%, from 20 to 50% in most cases. From the results, amitrole can be classified as "highly mobile" (Huhtanen, 1985b).

Table 6. Soil characteristics and Freundlich constants.

Soil	Freundlich constants		Organic content (%)	Correlation coefficient	K_{oc}	pH	CEC
	K_d	l/n					
California sandy loam	3.52	0.6487	3.0	0.9969	117	6.1	6
Plano silt loam	3.79	0.7739	5.9	0.9923	64.2	6.8	13
Kewaunee silty clay loam	1.57	0.8563	5.0	0.9990	31.4	7.0	17
Plainfield sand	0.685	0.7975	0.8	0.7525	85.6	5.6	1

In the same way, adsorption/desorption was investigated in four soils by equilibrating 12.5 g of soil with 10 ml of [3,5-^{14}C]amitrole solution at concentrations of 2 to 20 mg/l (Tables 7 and 8; Anderson and Hellpointner, 1989).

Table 7. Freundlich adsorption constants for soils at natural pH (5.3 to 7.4).

Soil	Constants		% organic carbon[1]	Correlation coefficient of the linear regression	K_{oc}	pH	CEC
	1/n	K_d					
Silty clay	0.7671	0.7141	0.64	0.9999	111.6	7.4	21.1
Sandy loam	0.8549	0.2231	0.75	0.9980	29.7	6.8	10
Sand	0.8722	0.1515	0.75	0.9993	20.2	5.6	4
Silt	0.8590	0.9215	1.8	0.9999	51.2	5.3	10.5

[1]Organic matter x 0.58

Table 8. Freundlich desorption constants for soils at natural pH (5.3 to 7.4).

	Constants	% organic carbon[1]	Correlation coefficient			

Soil	1/n	K_d		of the linear regression	K_{oc}	pH	CEC
Silty clay	0.9140	1.8078	0.64	0.9939	282.4	7.4	21.1
Sandy loam	0.9170	0.7839	0.75	0.9976	104.5	6.8	10
Sand	0.9321	0.7398	0.75	0.9982	98.6	5.6	4
Silt	0.8533	1.1986	1.80	0.9829	66.6	5.3	10.5

[1] Organic matter x 0.58

The investigation showed that 10.3 to 53.4% of the applied amitrole was adsorbed at equilibrium. There was no linear relationship between adsorption and the content of organic carbon or clay in the soils. The values for 1/n were <1 in all cases which corresponds to a decrease of adsorption with increasing concentration. Higher values were found for desorption than adsorption constants, with K_d values from 0.740 to 1.808 and K_{oc} from 66.6 to 282.4, and 24.8 to 72.3% of the adsorbed amitrole desorbed after single-stage desorption.

At a concentration of 20 mg adsorption to all the soils varied from pH 4.5 to pH 8, with differences in the amount adsorbed up to 46.5%. Increased adsorption was seen under acid conditions, where adsorption K_d values ranged from 0.575 to 2.279 and K_{oc} values from 76.7 to 356.2. The authors concluded that amitrole would be expected to show a high to very high mobility in soil at pH 5.3 to 7.4, and a medium to high mobility at pH 4.5 .

Mobility

The mobility of amitrole was compared with that of four reference compounds (refs) in selected soils by soil thin-layer chromatography (Table 9).

Table 9. Mobility of amitrole and four reference compounds in various soils.

Soil	% organic matter	Mobility class*				
		amitrole	Ref. 1	Ref. 2	Ref. 3	Ref. 4
Plainfield sand	0.8	5	5	5	5	1
California sandy loam	3.2	2	5	4	3	1
Dubuque silt loam	2.9	4	5	4	3	1
Hagerstown silty clay loam	2.5	3	4	3	3	1

* As defined by US EPA Pesticide Assessment Guidelines, Subdivision N, Page 67 (1982). 1 = Immobile (R_f = 0.0-0.09); 2 = Low mobility (R_f = 0.1-0.34); 3 = Intermediate mobility (R_f = 0.35-0.64); 4 = Mobile (R_f = 0.65-0.89); 5 = Very mobile (R_f = 0.90-1.0)

Amitrole and refs 2 and 4 were more mobile in Plainfield sand than in the other soils, while amitrole was generally less mobile than the standards (Huhtanen, 1985c).

In the above-mentioned field study (Biever, 1995), amitrole was rarely detected or quantified at any soil depth below 15 cm. It was quantified in one sample on day 89 in the 15 to 30 cm depth in Oregon and in two samples on day 61 at 15 to 30 cm in Washington.

Mobility of aged residues. [3, 5-^{14}C]amitrole was applied at 3.37 mg/kg dry soil to California sandy loam and incubated for 5 days at 25 ± 1°C. The aged soil was placed at the bottom of soil TLC plates which were developed in water. Amitrole is rapidly converted to carbon dioxide by wet, active soil. Aged amitrole residues are less mobile than unaged, probably because the carbon has been incorporated into soil or soil micro-organisms (Huhtanen, 1985d ; Table 10).

Table 10. Mobility of aged and unaged amitrole and standards in California sandy loam soil.

	Aged amitrole	Unaged amitrole	Ref. 1	Ref. 2
Rf	0.10	0.18	0.57	0.80
Mobility class	2	2	3	4

The mobility of amitrole and its degradation products was examined in two European soils. German standard soil 2.1 containing 0.6% organic carbon and 6.8% of the fraction <0.02 mm, and "Höfchen" soil containing 2.9% organic carbon and 32% of the fraction <0.02 mm. ^{14}C-labelled amitrole was used at an exaggerated concentration of 10 kg ai/ha, and duplicate tests were carried out after the soil had been incubated with amitrole for 0-30 and 92 days. The incubated soil was placed on top of 27 cm of untreated soil in glass columns and eluted with about 400 ml deionized water over 2 days.

More than 1% of the initially applied ^{14}C was leached from the German standard soil 2.1, 24-31% from the unaged soil, 1.5-1.9% from the 30-day and 0.7-1.6% from the 92-day soil. Most of the radioactivity in the 0-day leachate was from the parent compound (20-27%) but less than 0.1% of the residue after 30 days ageing. In the Höfchen soil, which had a higher content of organic matter, the radioactivity in the leachate decreased from 0.8% from the unaged soil to 0.1% from that aged for 92 days. Again, there was little leaching of fresh amitrole, and the aged residue was even less mobile (Weller, 1987).

Finally, in the field study by Biever (1995) cyanamide concentrations between 0.01 mg/kg and 0.02 mg/kg were measured in the 0 to 15 cm soil depth during the first 3 days at the Oregon site and were just detected from 0 to 21 days at the Washington site. Quantifiable concentrations of cyanamide were never found below 15 cm.

Lysimeter studies. In a lysimeter study conducted by Bayer A.G. with a grey-brown podzolic soil from loess, Ustinex KR (40% amitrole) was applied at 10 kg/ha (4 000 g/ha amitrole), twice a year from 1979 to 1983 and once a year from 1984 to 1986. Amitrole was not detected in the leachate at the limit of detection of 10 µg/l. Analyses were continued during 1988 and 1989 and no amitrole was detected at a limit of 0.05 µg/l (Bachlechner, 1988).

Environmental fate in water/sediment systems

Scholz (1995) investigated the aerobic degradation of amitrole in two water-sediment systems freshly sampled from fields in The Netherlands. Sediments were sieved to 2 mm and pre-incubated for 14 days in the ambient laboratory conditions. [3-^{14}C]amitrole was applied to the surface water at a rate equivalent to 5,000 g ai/ha. Both systems were analysed after 0, 3, 7, 14, 30, 60 and 91 days.

In both systems the recovery of ^{14}C exceeded 95% at all sampling times. The extent of mineralization was about 19% in one sediment and 10% in the other after 91 days. Radioactivity decreased continuously in water to 50% in one system and 36% in the other. The elimination half-lives in the total systems were 95 days and 76 days. Triazole and urea, both <3%, were the only identified products at day 7 in one system and at day 14 in the other. At least 4 other polar products (at the TLC origin) were observed, at a fairly constant total level of about 4-5%. Unextractable residues increased with time, reaching 27% and 40% of the applied radioactivity in the two sediments; but it was shown by vigorous hydrolysis that about 50% of this unextractable fraction was combined amitrole.

This study confirms the path followed by amitrole in biological media, the main difference being due to the continuous equilibrium between the aqueous phase where amitrole is mainly stable

and the sediment where it undergoes biological breakdown, and a reduced rate compared with aerobic soil degradation owing to the lower level of oxygen in the sediment.

These results were confirmed in another recent study (McCowan, 1995a) conducted for 52 weeks according to the US-EPA guideline. Analyses of surface waters and sediment extracts by thin-layer chromatography indicated that [^{14}C]amitrole was degraded over the incubation period, and accounted for about 5% of the applied radioactivity in raw samples and about 60% in sterile samples at the end of the study. A minor polar degradation product, with negligible mobility on the TLC plate, accounted for about 3% of the applied activity in non-sterile and about 7% in sterile samples. HPLC analysis indicated that this product probably consisted of 4 separate components, suggesting that this degradation pathway was partly abiotic. The half-life of amitrole at 22°C was in the same range as in the Scholz study at about 73-82 days, but was shorter (44 days) at 7°C.

The same aquatic system was used by McCowan *et al.* (1995b) to measure the rate of degradation of [^{14}C]amitrole over 52 weeks under anaerobic conditions at 22°C under sterile and non-sterile conditions and 7°C (non-sterile). The mean overall recoveries of applied radioactivity ranged from 96% to 105%. The volatile radioactivity, as $^{14}CO_2$ and organic volatiles, was very low throughout the incubation period in both experiments, accounting for less than 1% of the applied radioactivity. The radioactivity associated with the surface water at both temperatures decreased as the incubation progressed, accounting for about 74% of the applied radioactivity at 52 weeks. Extractable radioactivity in the sediment remained relatively constant throughout the incubation period, accounting for up to about 20% at 52 weeks. Bound residues were low (about 5% at 52 weeks) under both sterile and non-sterile conditions

Analysis by thin-layer chromatography indicated that [^{14}C]amitrole was the major radioactive component in surface waters and sediment extracts, accounting for about 84-87% of the applied radioactivity at 52 weeks in non-sterile samples and about 71% in sterile samples. A minor polar degradation product with negligible mobility on the TLC plate was detected in both sediment and water from 26 weeks onwards, accounting for about 4% of the ^{14}C at the end of the study. This was later postulated to be dehydrogenated amitrole (Williams and McGuire, 1997).

Amitrole → 3-Imino-1,2,4-triazole

In conclusion, [^{14}C]amitrole is degraded very slowly under anaerobic conditions, irrespective of temperature and biological activity. The half-life could not be calculated because of the limited degradation, but is more than one year.

METHODS OF RESIDUE ANALYSIS

Analytical methods

<u>Crops and soil</u>. A GLC method of analysis for the determination of amitrole residues in various plants and plant products was developed by Jarczyk and Möllhoff (1991). Fruits are extracted cold with ethanol/water (2:1). Grape must and wine are rotary-evaporated. The aqueous extract or

concentrate is purified by shaking with dichloromethane. Amitrole is acetylated with acetic anhydride and the derivative is partitioned into dichloromethane and cleaned up by gel permeation chromatography and column chromatography on silica gel before GLC determination with a thermionic nitrogen-phosphorus detector (NPD). The method has a routine limit of determination of 0.01 mg/kg with mean recovery levels of 82, 79, 98, 83, 77 and 76% for apples, pears, cherries, grapes, grape must and wine respectively.

Several important features are given special attention. (1) The analysis can only be interrupted after the acetylation of the extracts or standards. The final determination should be performed without delay because acetylated amitrole in solution is stable only for a short time even in a refrigerator. (2) Aqueous phases must not be evaporated to dryness and the water bath temperature must not exceed that specified. (3) Depending on the column and temperature programme used, GLC analyses may require 20-30 minutes between injections to allow time for interfering peaks from the previous samples to elute. (4) The peak areas of monoacetyl-amitrole on the capillary columns used are dependent on the sample matrix, so the derivative standard solution should be added to the cleaned-up extract from a control sample to yield a reference standard suitable for quantification.

An analytical method for blackberries was submitted by The Netherlands, in which amitrole is extracted with a mixture of ethanol and water, the extract is treated with hydrogen peroxide and interfering substances are removed by ion-exchange on both strongly and weakly acidic cation exchangers. Amitrole is converted to a complex with fluorescamine and determined by HPLC with fluorescence detector. The limit of determination is 0.02 mg/kg.

The GLC method of Jarczyk and Möllhoff (1991) for soil is essentially identical to their method for crops. The method has a routine limit of determination of 0.01 mg/kg with recoveries of 82-91%.

In addition to the points given special attention in the analysis of crops, the water content and the maximum water capacity of soil samples must be determined before the soil is extracted.

The HPLC method of Weber (1988) is applicable to crops, soil and water. It is described below.

More recently, the HPLC method was also adapted for determining residues of amitrole in grapes, wine and vine leaves (Clemson, 1995a,b,1966). Owing to difficulties encountered, a new method was developed for determining residues in plant material, which has been validated for use on grapes (McGuire, 1997) over the range 0.005-0.5 mg/kg. It involves extraction into acetone/water and dichloromethane partitioning. After acidification, the aqueous extract is cleaned up by solid-phase extraction and the eluate evaporated to dryness, resuspended in pyridine and derivatized with bis(trimethylsilyl)trifluoracetamide/trimethylchlorosilane (BSTFA/TMCS) before determination by GC-MS. GC-MS of amitrole standards over the range 0.01 to 5 µg/ml produced a linear calibration with a correlation coefficient of 0.997. The overall mean recovery of amitrole from fortified control grapes was 83.44%. This method is now being validated for must and wine, barley, wheat, peas and canola seeds.

Water. The HPLC method was developed by Weber (1988). Water samples are applied directly onto a cation exchange column, eluted with ammonia solution and further cleaned up by column chromatography on aluminium oxide. The HPLC determination is with ion pairing and electrochemical detection. The routine limit of determination is 0.1 µg/l with recoveries of 86-102%.

Products of animal origin. An HPLC method was validated by Weber (1997) is related to his 1998 method for crops, soil and water. Samples are extracted with water/acetone (3 :1), partitioned with dichloromethane and cleaned up on cation exchange and aluminium oxide columns. Amitrole is determined by HPLC with ion pairing and electrochemical detection. Mean recoveries at fortification levels of 0.01 mg/kg to 0.1 mg/kg were 74% for milk, 82% for eggs and 87% for muscle. The limit of detection was 0.005 mg/kg and the LOD 0.01 mg/kg. At this level the recovery was 81% from muscle.

GLC methods have been adapted from those used for fruit, soil and water (Jarczyk, 1981, 1982a, 1984). They involve extraction with ethanol/water (2/1), clean-up by partition with organic solvents such as carbon tetrachloride or chloroform, derivatization with acetic anhydride, and further clean-up by column chromatography on silica gel. Amitrole is determined as the monoacetyl derivative by GLC with a thermionic nitrogen-phophorus detector (NPD).

These methods have been validated at various fortification levels over a long period by many analysts. The recoveries are usually between 70 and 110% with a relative standard deviation below 20% and blank values below 30% of the limit of quantification. The limits of quantificaton differ between methods and samples, but are in the range 0.01-0.025 mg/kg.

Stability of residues in stored analytical samples

In a frozen storage stability study on grapes (Clemson, 1997) untreated grapes were dipped into a solution of amitrole at 0.28 mg/kg. After two years at -20°C, the residues were about 60% of the initial level. A study on apples by Biever is in progress. Residues after 16 months were about 75% of the initial levels.

USE PATTERN

Besides uses on industrial land, roadsides, railways, ditches etc. amitrole is used world-wide as a herbicide in vineyards and orchards against all kinds of weeds (grasses and dicotyledons, annual, biannual and perennial), and also as a total weed killer after harvest and before the next annual sowing. Table 11 shows the recommended uses of amitrole on apples, pears, peaches and grapes.

Table 11. Use patterns of amitrole.

Crop	Country	Formulation	Application			PHI, days
			Number	kg ai/ha	kg ai/hl	
Fruit trees	Australia	SL	2	1.4-2.8	0.14-0.28	56
	Belgium	SL		3.6-4.8	0.9-1.2	
	France	SL		2.4-4.8		
	Germany	WG	1	3	0.3-0.75	60
	Morocco	SL	1-2	2.4-7.2	0.24-1.4	
	Spain	SL		1.92-3.84	0.48	45
Vines	Australia	SL	2	1.2-2.4	0.14-0.28	56
	Germany	WG	1	3	0.3-0.75	60
	France	SL		2.4-4.8		
	Morocco	SL	1-2	2.4-7.2	0.24-1.4	
	Spain	Sl		0.192-0.384	0.048	45

RESIDUES RESULTING FROM SUPERVISED TRIALS

Apples. Thirty four trials were conducted in Europe. In the eighteen trials in France according to GAP the residues from day 5 to day 171 were <0.01 mg/kg. In fourteen trials in Germany above the GAP rate the residues after 4 to 120 days were <0.02 mg/kg. In two trials in Portugal above reported European GAP rates the residues after 111 days were <0.01 mg/kg. The samples were stored at -20ºC for 56 to 483 days before analysis.

Table 12. Results from residue trials with amitrole on apples.

COUNTRY Report No Year	Formulation	Application			PHI days	Residues, mg/kg	Reference
		No.	kg ai/ha	kg ai/hl			
FRANCE[1] 95-08-6065 1993	SL	2-4	2.88	0.576	62 116 151 172	<0.01 <0.01 <0.01 <0.01	
	SL	2-4	4.80	0.960	62 116 151 172	<0.01 <0.01 <0.01 <0.01	
	SL	2-4	2.88	0.576	31 85 120 141	<0.01 <0.01 <0.01 <0.01	
	SL	2-4	4.80	0.960	31 85 120 141	<0.01 <0.01 <0.01 <0.01	
	SL	2-4	2.88	0.576	54 89 110	<0.01 <0.01 <0.01	
	SL	2-4	4.80	0.960	54 89 110	<0.01 <0.01 <0.01	
	SL	2-4	2.88	0.576	5 56	<0.01 <0.01	
	SL	2-4	4.80	0.960	35 56	<0.01 <0.01	

COUNTRY Report No Year	Formulation	Application			PHI	Residues,	Reference
		No.	kg ai/ha	kg ai/hl	days	mg/kg	
	SL	2-4	2.88	0.576	61 101 150 171	<0.01 <0.01 <0.01 <0.01	
	SL	2-4	4.80	0.960	61 101 150 171	<0.01 <0.01 <0.01 <0.01	
	SL	2-4	2.88	0.576	40 89 110	<0.01 <0.01 <0.01	
	SL	2-4	4.80	0.960	40 89 110	<0.01 <0.01 <0.01	
	SL	2-4	2.88	0.576	49 70	<0.01 <0.01	
	SL	2-4	4.80	0.960	49 70	<0.01 <0.01	
	SL	2-4	2.88	0.576	58 92 119	<0.01 <0.01 <0.01	
	SL	2-4	4.80	0.960	58 92 119	<0.01 <0.01 <0.01	
	SL	2-4	2.88	0.576	34 61	<0.01 <0.01	
	SL	2-4	4.80	0.960	34 61	<0.01 <0.01	
GERMANY 0625-79 1979	WP	2	4	0.666	50 70 90	<0.02 <0.02 <0.02	
0626-79	WP	2	4	0.666	50 70 90	<0.02 <0.02 <0.02	
0627-79	WP	2	4	0.4	50 70 90	<0.02 <0.02 <0.02	
0637-79	WP	2	4	0.666	55 79 98	<0.02 <0.02 <0.02	
0600-82 1982	WP	2	4	0.666	6 36 56 96	<0.02 <0.02 <0.02 <0.02	
0601-82 1982	WP	2	4	0.666	4 34 64 93	<0.02 <0.02 <0.02 <0.02	
0726-90 1990	WP	1	4	1	42	<0.01	
0727-90	WP	1	4	1	42	<0.01	
0600-83 1983	WP	1	15	3.75	60 90 120	<0.02 <0.02 <0.02	
0601-83	WP	1	15	3.75	60 90 120	<0.02 <0.02 <0.02	
0602-83	WP	1	15	3.75	60 90 120	<0.02 <0.02 <0.02	

COUNTRY Report No Year	Formulation	Application			PHI	Residues,	Reference
		No.	kg ai/ha	kg ai/hl	days	mg/kg	
RA-200391	WP	2	5	0.833	11	<0.01	0514-91
	WP	2	5	0.833	50	<0.01	0515-91
	WP	2	5	0.833	26	<0.01	0516-91
PORTUGAL RA-2004	WP	1	6	0.6	111	<0.01	0229-92
1991	WP	1	6	0.6	111	<0.01	0230-92

[1]The number of applications varied from 2 to 4, but the number of applications in each trial was not clear

Pears. In one trial in Portugal and three trials in Germany, at higher rates than German and other European GAP, residues after 60 to 120 days were below the LOD (0.01 or 0.02 mg/kg).

Table 13. Results from trials with amitrole on pears after 1 application of a WP formulation.

COUNTRY Report No Year	Application			PHI, Days	Residues, mg/kg
	N°	kg ai/ha	kg ai/hl		
PORTUGAL 0231-92 1992	1	6	0.6	81	<0.01
GERMANY 0603-83 1983	1	15	3.75	60 90 108	<0.02 <0.02 <0.02
0604-83	1	15	3.75	60 90 120	<0.02 <0.02 <0.02
0605-83	1	15	3.75	60 90 120	<0.02 <0.02 <0.02

Peaches. In four trials with amitrole in Spain with 1 application of 6 kg ai/ha (0.6 kg ai/hl) of WP formulation which corresponds to nearly twice the GAP rate, residues in the fruit without stone were <0.01 mg/kg at PHIs of 42 to 105 days. The GAP PHI is 45 days.

Grapes. In twenty four trials in France and sixteen in Germany at GAP rates or higher, the residues in all but one trial were below the LOD (0.02 mg/kg).

Table 14. Results from trials on grapes in France and Germany with SL and WG formulations.

COUNTRY Report No Year	Application			PHI days	Residues mg/kg
	N°	kg ai/ha	kg ai/hl		
FRANCE 94/CPF017/0450 1992	1	2.8	0.576	102 137 165 179	0.087[1] <0.02 <0.02 <0.02
	1	4.8	0.960	102 137 165 179	≤0.02 <0.02 <0.02 <0.02
	1	2.8	0.576	20 55 83 97	≤0.02 <0.02 <0.02 <0.02
	1	4.8	0.960	20 55	≤0.02 <0.02

COUNTRY Report No Year	Application			PHI days	Residues mg/kg
	N°	kg ai/ha	kg ai/hl		
				83 97	<0.02 <0.02
	1	2.8	0.576	35 63 77	<0.02 <0.02 <0.02
	1	4.8	0.960	35 63 77	<0.02 <0.02 <0.02
	1	2.88	0.576	98 129 158 178	<0.02 <0.02 <0.02 <0.02
	1	4.80	0.960	98 129 158 178	<0.02 <0.02 <0.02 <0.02
	1	2.8	0.576	29 60 89 109	<0.02 <0.02 <0.02 <0.02
	1	4.80	0.960	29 60 89 109	<0.02 <0.02 <0.02 <0.02
	1	2.8	0.576	31 60 80	<0.02 <0.02 <0.02
	1	4.80	0.960	31 60 80	<0.02 <0.02 <0.02
	1	2.8	0.576	94 126 146 175	<0.02 0.03 <0.02 <0.02
	1	4.80	0.960	94 126 146 175	<0.02 <0.02 <0.02 <0.02
	1	2.8	0.576	41 73 93 122	<0.02 <0.02 <0.02 <0.02
	1	4.80	0.960	41 73 93 122	<0.02 0.03 <0.02 <0.02
	1	2.8	0.576	18 50 70 99	<0.02 <0.02 <0.02 <0.02
	1	4.80	0.960	18 50 70 99	<0.02 <0.02 <0.02 <0.02
	1	2.8	0.576	113 147 168 189	0.03 <0.02 <0.02 <0.02
	1	4.80	0.960	113 147 168	<0.02 <0.02 <0.02

COUNTRY Report No Year	Application			PHI days	Residues mg/kg
	N°	kg ai/ha	kg ai/hl		
				189	<0.02
	1	2.8	0.576	43 77 98 119	<0.02 <0.02 <0.02 <0.02
	1	4.80	0.960	43 77 98 119	<0.02 <0.02 <0.02 <0.02
	1	2.8	0.576	15 49 70 91	<0.02 <0.02 <0.02 <0.02
	1	4.8	0.960	15 49 70 91	<0.02 <0.02 <0.02 <0.02
GERMANY 0634-79 1979	2	4	0.666	60 80 100 120	<0.02 <0.02 <0.02 <0.02
0635-79	2	4	0.666	60 80 100 120	<0.02 <0.02 <0.02 <0.02
0636-79	2	4	0.666	60 78 100 120	<0.02 <0.02 <0.02 <0.02
0606-83	2	4	1	80	<0.02
0607-83	2	4	1	80	<0.02
0600-85	2	4	1	28 35 42 49	<0.02 <0.02 <0.02 <0.02
0601-85	2	4	1	28 35 42 49	<0.02 <0.02 <0.02 <0.02
0602-85	1	4	40	28 35 42 49	<0.02 <0.02 <0.02 <0.02
0603-85	1	4	1	28 35 42 49	<0.02 <0.02 <0.02 <0.02
0604-85	2	4	1	28 42 56 63	<0.02 <0.02 <0.02 <0.02
0605-85	2	4	1	28 42 56 63	<0.02 <0.02 <0.02 <0.02
0724-90 1990	1	4	1	42	<0.01
0725-90	1	4	1	43	<0.01
0252-91 1991	1	4	1	75	<0.01
0253-91	1	4	1	75	<0.01

COUNTRY Report No Year	Application			PHI days	Residues mg/kg
	N°	kg ai/ha	kg ai/hl		
0254-91	1	4	1	56	<0.01

[1]High residue was found to be a result of cross contamination between trials

NATIONAL MAXIMUM RESIDUE LIMITS

The following national MRLs were reported.

Country	Commodity	MRL, mg/kg
Australia	Citrus fruits, pome fruits, grapes	0.01
	Stone fruits	0.02
	Cereal grains	0.01
	Potato	0.05
	Pecan nut	0.01
	Sugar cane	0.01
Canada	Maize, soya beans, white beans, apples	no MRL (no residue > 0.1 mg/kg)

APPRAISAL

Amitrole was first considered by the JMPR in 1974. It was re-evaluated in 1993 within the CCPR Periodic Review Programme, and a temporary ADI was allocated. A full ADI was allocated in 1997. No MRLs have been established, but it is stated that uses of amitrole should be restricted to those where residues in food would not be expected to occur. The 1993 Meeting recommended a further note that “A realistic limit of determination for the general monitoring of amitrole would be 0.05 mg/kg”. This evaluation is within the CCPR Periodic Review Programme. New data on metabolism in rats and environmental fate, in addition to residue trials on apples, pears, peaches and grapes were reported by the manufacturer. The governments of The Netherlands and Poland provided information on analytical methods and national MRLs.

In addition to studies submitted previously to the JMPR, two recent studies (1995 and 1996) on metabolism in mice and rats were submitted. When [5-^{14}C]amitrole was administered orally to rats, absorption from the gastrointestinal tract was rapid and the peak plasma level was reached after 40 to 60 minutes in both dose groups (1 and 500 mg/kg bw). When the animals were killed 48 hours after administration, radioactivity was detected mainly in the liver and to a small extent in the kidney cortex and nasal mucosa. Biotransformation to volatile metabolites including carbon dioxide was negligible. More than 97% of the recovered radioactivity was excreted within 48 or 72 hours after oral administration. The major route of elimination (91 to 98%) was renal. The half-life of elimination was shorter after intravenous than after oral administration. Amitrole accounted for more than 86% of the identified radioactivity and for about 66% or more of the administered dose. Small amounts of 7 metabolites were detected. The proposed pathway of biotransformation, mainly in the liver, is *via* substitution at C-5 by *N*-acetylcysteine to form 3-amino-1,2,4-triazolyl-*5*-mercapturic acid, which is hydrolysed to 3-amino-5-mercapto-1,2,4-triazole and excreted either directly or after methylation to 3-amino-5-methylthio-1,2,4-triazole.

Amitrole is a fast-acting herbicide which is taken up predominantly through the leaves. The only significant difference between the metabolism of amitrole in rats and in plants is the production of triazolylalanine (3-(1,2,4-triazol-l-yl)-2-aminopropionic acid) in plants. In a study on apples, triazolylalanine was the major metabolite (22-24%), occurring in the free form and as conjugates. This compound is also produced by the metabolism of other triazole pesticides and was therefore reviewed by the 1989 JMPR, which concluded that residues of triazolylalanine do not

present a toxicological hazard. The ^{14}C balance at harvest after 5 months showed 1.11% in the tree, 0.07% in the harvested fruit, 0.16% in the roots and 42% remaining in the soil.

No metabolism studies were reported on farm animals.

In addition to studies which have been evaluated previously by the JMPR, new studies on the environmental fate in soil, water and sediment were reported. In soils, amitrole is rapidly degraded to NH_3 and CO_2, in addition to cyanamide and urea. In laboratory studies, the half-life of amitrole ranged from 2 to 26 days at ambient temperature in different soils. Adsorption-desorption studies showed significant adsorption and desorption, with K_{OC} values varying with the soil type from 20 to 120. It therefore appears that amitrole would readily be leached, but in leaching studies of soil with fresh or aged residues, as well in the lysimeter studies, leaching was not observed. This can be attributed to adsorption to the soil which, although reversible, gives time for breakdown to occur and prevents the compound from being leached to groundwater. This was confirmed by a field study in which amitrole was rarely detected below 15 cm. Therefore under practical conditions there is little risk that amitrole would reach the groundwater level.

The aerobic degradation of amitrole was investigated in 2 water-sediment systems, freshly sampled from fields in The Netherlands. mineralization amounted to 19 and 10% after 91 days and the half-lives were 95 and 76 days. Triazole and urea, both <3%, were the only identified products and at least 4 other polar compounds were observed at levels of about 4-5%. Unextractable residues increased with time, but it was shown by reverse isotope dilution analysis that approximately 50% of the unextractable fraction was still unchanged amitrole. In another study under anaerobic conditions at 22°C amitrole was also the major component present, accounting for about 71-87% of the applied radioactivity, after 52 weeks.

In a method of analysis for the determination of amitrole residues in various plants and plant products cold extraction with ethanol/water is followed by partition with dichloromethane and acetylation of the amitrole. The derivative is cleaned up by gel permeation and silica gel chromatography. The limit of determination is 0.01 mg/kg and recoveries ranged from 76 to 98%. The same method can be applied to soil, with a limit of determination of 0.01 mg/kg and recoveries of 82-91%. A method for blackberries using HPLC with fluorescence detection after conversion to a complex with fluorescamine was reported by the government of The Netherlands. The LOD is 0.02 mg/kg and recoveries range from 103 to 127%.

In an HPLC method with EC detection for the determination of amitrole residues in water, the sample is applied directly to a cation exchange column which is eluted with ammonia solution, and the eluate is further cleaned up on an aluminium oxide column. The limit of determination is 0.1 µg/l with recoveries in the range 86-102%. HPLC with electrochemical detection can also be used for samples of animal origin, after extraction with water/acetone, partition with dichloromethane and clean-up on ion-exchange and aluminium oxide columns. Mean recoveries at fortification levels of 0.01 to 0.1 mg/kg varied from 74% for milk to 87% for muscle.

A method in which extraction with acetone/water is followed by partitioning with dichloromethane was validated for grapes over the range 0.005-0.5 mg/kg. After acidification, a portion of the aqueous extract is cleaned up by solid-phase extraction, the eluate is evaporated to dryness, the residue is re-suspended in pyridine and the amitrole is derivatized with bis(trimethylsilyl)trifluor-oacetamide/trimethylchlorosilane (BSTFA/TMCS) before determination by GC-MS. The overall mean recovery was 83%.

The stability of residues in stored analytical samples was determined in grapes and apples at -20°C, after spiking with amitrole at levels from 0.05 to 1 mg/kg. The residues after 16 to 24 months were 60 to 75% of the initial levels.

On the basis of the animal and plant metabolism studies, the definition of the residue for both enforcement and the estimation of dietary intake should be amitrole.

Residues resulting from supervised trials

In eighteen trials on apples at the GAP rate (no specified PHI) in France, fourteen trials above the GAP rate (60 days PHI) in Germany, and two trials in Portugal where there is no GAP, the residues in the fruit from day 4 to day 171 were below the LOD (0.01 or 0.02 mg/kg). In one trial with pears in Portugal (no GAP) and three in Germany at a higher rate than recommended GAP, the residues after 60 to 120 days were similar. In four trials with peaches in Spain at twice the GAP rate (no PHI), the residues in the fruit without stones were <0.01 mg/kg at PHIs from 42 to 105 days.

As the reported GAP covers stone and pome fruits and the residues were determined over a wide range of PHIs, the Meeting estimated a maximum residue level at a practical limit of determination of 0.05 mg/kg and an STMR of 0 for amitrole in pome fruits and stone fruits. The residues of amitrole in fruit below the LOD are consistent with the results of the metabolism studies on apples.

In twenty four trials on grapes in France according to GAP (no PHI) the residues were <0.02 (20), 0.03 (3) and 0.087 mg/kg. The high value of 0.087 mg/kg was reported to be due to contamination from an adjacent trial and was ignored. Sixteen trials in Germany at a higher rate than the recommended GAP (60 days PHI) yielded residues in fruit after 15-189 days below the LOD (0.02 mg/kg).

The Meeting estimated a maximum residue level of 0.05 mg/kg and an STMR of 0.02 mg/kg for amitrole in grapes.

DIETARY RISK ASSESSMENT

STMRs were estimated for amitrole in pome fruits, stone fruits and grapes. As no other MRLs or STMRs have been previously established for amitrole these were the only ones used in the estimates of dietary intake. The International Estimated Daily Intakes for the five GEMS/Food regional diets were 0% of the ADI. The Meeting concluded that the intake of residues of amitrole resulting from its uses that have been considered by the JMPR is unlikely to present a public health concern.

RECOMMENDATIONS

The Meeting estimated the maximum residue levels and STMR levels shown below. The maximum residue levels are recommended for use as MRLs.

Definition of the residue for enforcement and estimation of dietary intake: amitrole.

CCN	Commodity	MRL, mg/kg	STMR, mg/kg
FS 0009	Pome Fruit	0.05*	0
FS 0012	Stone Fruit	0.05*	0
FB 0269	Grapes	0.05	0.02

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