AMITROLE (079)

EXPLANATION

Amitrole (3-amino-1*H*-1,2,4-triazole) was originally evaluated by the JMPR in 1974. A conditonal ADI was established at 0-0.00003 mg/kg bw, and a conditional MRL for amitrole in raw agricultural commodities of plant origin was recommended at the limit of determination of 0.02 mg/kg. The ADI was confirmed by the JMPR in 1977, but the 17th Session of the CCPR (1987) recommended that the CXL (as it had become) at the limit of determination should be deleted and replaced by the statement that uses of amitrole should be restricted to those where residues in food would not be expected to occur.

The compound was scheduled for re-evaluation by the 20th Session of the CCPR (1990), and information on GAP was requested by a circular letter in 1991.

The Meeting was supplied with information on registered uses in several, mostly European, countries but data from residue trials were received only on grapes. The Meeting was provided with information on the fate of amitrole in animals, plants and soil, and on new analytical methods for the determination of amitrole in plant material, soil and water.

IDENTITY

Physical properties

The octanol/water partition coefficient was determined to be 0.108.

USE PATTERN

Information was supplied to the Meeting on registered uses of amitrole in eight countries (Table 1). Amitrole is used also on industrial land, roadsides, railways, ditches etc. Such uses are not quoted in the Table. In the 1974 evaluation detailed descriptions were given of the uses of amitrole on many crops and of non-agricultural uses. In all cases the compound should be applied in such a way that no residues are detectable in food commodities.

Table 1. Registered uses of amitrole

Country	Crop	Application			PHI, days
		No.	Rate, kg ai/ha	Spray conc., kg ai/hl	

Country	Crop	Application			PHI,
		No.	Rate, kg ai/ha	Spray conc., kg ai/hl	days
Australia	Vines and fruit	6-8 week intervals		0.14-0.28	
	Potatoes		1.4-2.8		28-35
	Cereals		0.7-1.4	0.58	sowing after > 5 days
	Pasture		0.75-1.0	0.58	graze after > 6 months
Belgium	Apple		3.0-5.0		
	Pear		5.0		
Canada	Apple orchards	1	to cover ground: 1.2 bands round trees: 0.03		> 60
		> 1	directly on ivy leaves: 2.2-4.6		30
	Maize Soya beans White beans	1	2.2-4.6		pre-sowing
	Cereals, Peas, Alfalfa, Clover	1	3.4-5.5		8 months
	Pasture	2	7.5-15		6 months
France	Apple, Pear	1	1.8		
	Other fruit	1	5.0		
Germany	Pome fruit	2	1.5-2.0		42
		1	5.0		in spring
	Plum	2	2.0		42
	Cherry	2	2.0		42
	Grape	1	2.0		42
Netherlands	Apple Pear	1-2	3.0-4.0		before blossom or after harvest
	Plum Cherry Currants	1	3.0-4.0		after harvest before 1 Nov.
Portugal	Grape	1	1.9-2.5		
Spain	Citrus fruits	1	2.5-6.0		post- and pre-emergence of weeds
	Pome fruit	1-2	2.5-6.0		as for citrus
	Grape	1	1.5-3.6		as for citrus
	Hazelnut	1	2.5-5.4		as for citrus
	Olive	1	2.4-3.2		as for citrus

RESIDUES RESULTING FROM SUPERVISED TRIALS

The Meeting was informed of one report on completed trials with amitrole on grapes. New trials are being carried out on apples, pears and grapes but have not yet been completed. It was stated that reports on most of this work, including the study on grapes, would be supplied in the near future.

FATE OF RESIDUES

In the 1974 evaluation numerous studies on the degradation of amitrole in plants and soil were reviewed, but in some areas with some uncertainty about the extent of degradation and, especially in soil, about whether degradation is mainly a microbiological or a chemical process.

Several reports from new studies on the degradation of amitrole in animals, plants and soil were provided to the Meeting.

In animals

The metabolism of amitrole in rats was investigated and compared with its degradation in beans. Unaltered amitrole and three metabolites were present in the urine of treated animals, the metabolites being similar to those in beans. One of the major metabolites in rats was compatible with the structure of aminotriazolylalanine, 3-(3-amino-1,2,4-triazol-1-yl)-D-alanine, which had previously been identified as the major metabolite in plants (Franco and Municio, 1975).

In plants

In the evaluation of the 1974 JMPR several investigations of the fate of amitrole in plants were reviewed with the main conclusions that little or none of the compound is taken up from the soil via the roots to the leaves and fruit, and that if application is made directly to the leaves most of the absorbed compound is metabolized. Three or four metabolic products were observed, but only one was identified (as aminotriazolylalanine).

Further investigations of the possible uptake from soil to plant and of the metabolism in plants have been carried out.

In a study of the metabolism of some herbicides including amitrole by Field Horsetail (*Equisetum arvense*), 5-^{14}C-labelled amitrole sprayed on the plant showed evidence of conjugation in the shoots and rhizomes. In the shoots the main compound was the parent (>76%), together with aminotriazolylalanine and an unidentified metabolite. In the rhizomes the proportion of intact amitrole was lower. No further metabolites were identified. The quantity of aminotriazolylalanine decreased with time, with an increase of the unidentified metabolites.

This study also showed that conjugates with low mobility in the vascular system were formed (Marschall *et al.*,1987).

A report from Bayer AG (1982) on the degradation of amitrole in plants and soil describes experiments with treatments of *Ranunculus repens*, grapes and apples, the grapes and apples being grown in pots.

In the experiment with direct application to the weed (*Ranunculus*) 80% of the amitrole was metabolized within a few days, and the compounds were mainly present in the ends of the shoots.

For apples the soil surrounding the trees was treated with ^{14}C-labelled amitrole (about 4 kg ai/ha) twice a year for three years. The apples contained 0.1% of the radioactivity applied (about 0.2 mg/kg calculated as amitrole) in the first year of application. The level decreased to 0.02% in the second year. Approximately 18% of this was found to be the parent compound, at about 0.03 mg amitrole/kg.

A 3-year experiment was also carried out on grapes, where [^{14}C]amitrole was applied to the soil at a rate used in practice. After 90 days 1-2% of the applied radioactivity was found, mainly in the leaves. Radioactivity was present in the woody part of the vines 2 years after the last application, although in reduced quantities. The concentration of radioactivity in the grapes was approximately 0.2% of that applied during the last year of treatment. No radioactivity was detectable in the grapes after 2 years (Bayer AG, 1982).

In other experiments on the metabolism of amitrole in apples 3,5-[^{14}C]amitrole was applied to the soil in which apple trees were grown under outdoor conditions in tubs, to excised sprouts from apple trees, and to cell suspension cultures. Mature fruit from the outdoor experiment contained at most 0.05 mg/kg total residues, 75% water-soluble and 25% bound to insoluble material. No parent compound was detectable in the fruit. The major metabolite (maximum 0.012 mg/kg) was triazolylalanine (3-(1,2,4-triazol-1-yl)-D-alanine), which was present in the free form and as conjugates. More than 50% of the radioactivity was reassimilated ^{14}C incorporated into natural plant constituents.

In the tub experiment one apple tree absorbed 1.1% of the radioactivity applied to the soil, 0.07% was found within the mature fruit and about 42% remained in the soil after 5 months. In contrast to this the major metabolite in model experiments with excised apple sprouts and cell suspension cultures was not triazolylalanine but aminotriazolylalanine, although in excised sprouts small amounts of triazolylalanine were also present. In cell suspension cultures at high concentrations of amitrole, 3,5-dihydroxy-1,2,4-triazole was the main compound found (Schneider *et al.*, 1991).

In soil

The 1974 Meeting reviewed several reports on the degradation

of amitrole in soil. According to these degradation is rapid and the main product is CO_2. Some disagreement was obvious in the conclusions of these reports as to what extent degradation in soil is microbiological or chemical.

Since 1974 several experiments have been carried out on the degradation of amitrole in soil. In one, degradation and translocation were examined in sandy and loamy soils under northern German climatic conditions. Because of adsorption and rapid degradation amitrole was practically not translocated, but the possibility nevertheless exists of contamination of groundwater in sandy soils with a low content of organic material and a high water level (Drewes and Blume, 1976).

The degradation of amitrole was studied in the laboratory in two types of soil, an English loam and the German standard soil 2.2. Amitrole was labelled with ^{14}C in the 3 and 5 positions, and the soil was kept in darkness during the experiment. It was shown that the conditions exerted a marked influence on the degradation. Under aerobic conditions amitrole was extensively degraded to $^{14}CO_2$. After 28 days the production of $^{14}CO_2$ in German standard soil 2.2 amounted to 70-80% of the applied radioactivity, and in English loam soil to 40-50%, but in strictly anaerobic soil no volatile radioactivity was produced. In the aerobic soil most of the radioactivity which did not appear as $^{14}CO_2$ was not extractable and the major extractable radioactive compound was unchanged amitrole. No intermediates in the degradation to $^{14}CO_2$, including urea and cyanamide which were observed in other experiments, were detected in the soil extracts. In anaerobic soil the total non-bound radioactivity decreased to 60% of that applied after 28 days and to 25% after one year.

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

The degradation of ^{14}C-labelled amitrole was also studied in a field experiment. Field plots of loamy soil were treated with the labelled compound at 20 mg/plot (about 20 kg ai/ha) and exposed to the prevailing weather conditions.

Immediately after application the recovery of applied radioactivity was 97%, which decreased to 75% after 56 days and 53% after 112 days. Most of the radioactivity was in the top 5 cm layer of the soil at all times. After 112 days 43% of the applied radioactivity was present in this layer, about 6% in the 5-15 cm layer and about 4% in the 15-30 cm layer.

90% of the radioactivity in the extracts from the top 5 cm layer of soil was present as a compound corresponding to unchanged ^{14}C-amitrole at all sampling times. The level of ^{14}C-amitrole in the top layer declined from 22 mg/kg shortly after application to 3 mg/kg after 56 days and <0.1 mg/kg after 112 days (Hawkins *et al.*, 1982b).

A laboratory experiment was carried out on the degradation of amitrole in soil with the aim of identifying the intermediate compounds involved. Amitrole was labelled at the 3 and 5 positions. The soil was a clayish silt, which 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 degradaded to CO_2. The residue in the extract of the soil consisted mostly of the parent compound, with <2.5% as metabolites. 5-hydroxy-amitrole was the primary metabolite, but was degraded very rapidly. Cyanamide and urea were also found as degradation products. Uracil presumely represents a secondary pathway in the metabolism. In the proposed degradation scheme the ring is opened between positions 1 and 5 after 5-hydroxy-amitrole is formed, 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 (Scholz, 1988).

In water

The stability of amitrole was studied in buffer solutions at 90 C at pH 4, 7 and 9 and a concentration of about 10 mg/l. Samples were taken 0, 19, 94 and 114 days after application. No degradation was observed, indicating that the half-life of amitrole in water at 20 C would be more than one year (Krohn, 1986).

Leaching

The mobility of amitrole and its metabolites was examined in two soil types: German standard soil 2.1 containing 0.6% organic carbon and 6.8% of the fraction <0.02 mm, and "Höpfchen" soil containing 2.9% organic carbon and 32% of the fraction <0.02 mm. ^{14}C-labelled amitrole was used at a concentration similar to that applied in practice of 10 kg ai/ha, and the tests were carried out after the soil had been incubated with amitrole for 0, 30 and 92 days, each test in duplicate. Incubated soil was placed on top of 27 cm of untreated soil in glass columns. A total of about 400 ml deionized water was used as the eluant over 2 days.

Only in the German standard soil 2.1 was more than 1% of the initially applied ^{14}C detected in the leachate. The percentage leached decreased from 24-31% from the 0-day soil to 1.5-1.9% from the 30-day and 0.7-1.6% from the 92-day. Most of the radioactivity in the 0-day leachate was from the parent compound (20-27%). Less than 0.1% of the parent compound was detected in the residue after 30 days aging. In the "Höfchen" soil, which had a considerably higher content of organic matter, the radioactivity in the leachate decreased from 0.8% from the 0-day soil to 0.1% from the soil aged for 92 days (Weller, 1987).

METHODS OF RESIDUE ANALYSIS

Analytical methods recorded in the evaluation of the 1974 JMPR were all colorometric, usually after derivatization of amitrole in cleaned up extracts. Several of them were modifications of an FDA method developed by Storherr and Burke (1961).

Gas-chromatographic methods have now been developed, using a nitrogen-specific detector, for the determination of residues of amitrole in apples and pears and in soil and water. An ethanol/water mixture is used for extraction, and after evaporation of the solvent the residue is acetylated with acetic anhydride and transferred to chloroform. Clean-up is by column chromatography on silica gel with ethyl acetate as eluant. The limit of determination in fruit and soil is 0.02 mg/kg and in water 0.002 mg/kg (Jarczyk, 1982, 1983).

A method has been developed to determine amitrole in water by TLC. After evaporation of water the residue is cleaned up by column chromatography on silica gel using an acetonitrile/NH_3/methanol mixture as eluant. Amitrole is detected on the chromatogram by diazotization and coupling to form an azo compound. Quantification is by densitometry. The **limit of determination is 0.05 ìg/l (Burger, 1986).**

HPLC methods have also been developed for the determination of residues of amitrole. In one method residues in vegetables are determined using the extraction and clean-up procedures described by Storherr and Burke (1961). The residue is then diazotized and cleaned up by column chromatography on polyamide and ion-pair HPLC. The limit of determination for residues in potatoes and beets was 0.01 mg/kg (Løkke, 1987).

Another HPLC method was developed to determine residues in blackberries. Residues are extracted with ethanol/water and, after pre-treatment with hydrogen peroxide, interfering substances are removed by ion exchange on an acidic cation-exchanger. The residue is then converted to an amitrole-fluorescamine complex and determined by HPLC with fluorescence detection. The limit of determination is 0.02 mg/kg (Dornseiffen and Verwaal, 1988).

A third HPLC method has been developed for the determination of residues in plant material, soil and water. Amitrole is extracted with acetone/water and organic components are separated by partitioning with dichloromethane. Amitrole is then isolated on a cationic ion-exchanger and purified by column chromatography on alumina. The residue is determined by HPLC with electrochemical detection. The limit of determination in vegetables is 0.01 mg/kg, in soil 0.005 **mg/kg, and in water 0.1 ìg/l (Weber, 1988).**

NATIONAL MAXIMUM RESIDUE LIMITS

National maximum residue limits reported to the Meeting are shown below.

Country	Crop	MRL, mg/kg

Australia	Avocados, bananas, cereal grains, citrus, grapes, meat, milk, milk products, passionfruits, paw-paws, pecans, pineapples, pome fruit, sugar cane	0.01
	stone fruit	0.02
	potatoes	0.05
	water	0.001
Netherlands	Agricultural food commodities	0.05 (limit of determination)
Spain	Citrus fruit, grapes, grapes, hazelnuts, Pome fruit, olives	0.05 (limit of determination)

APPRAISAL

Amitrole was evaluated by the JMPR in 1974 and 1977 and is included in the CCPR periodic review programme. A conditional ADI was allocated in 1974 and confirmed in 1977. An MRL for raw agricultural commodities was recommended at the limit of determination in 1974, but the 17th Session of the CCPR (1987) recommended that the MRL should be withdrawn and replaced by a note that uses of amitrole should be restricted to those where residues in food would not be expected to occur.

Information on registered uses was received from Australia, Belgium, Canada, France, Germany, The Netherlands, Portugal and Spain. The compound is applied to the ground and directly on to weeds and usually with a long PHI, so residues should not be detectable in crops grown on treated soil.

The Meeting received only one report from supervised trials, but was informed that new trials on apples, grapes, and pears were in progress. Most of the studies would be supplied to the JMPR in the near future.

Several reports were available from studies on the metabolism or degradation of amitrole (aminotriazole) in plants, animals and soil. In plants after direct applications to the leaves or stem the main metabolite was aminotriazolylalanine, 3-(3-amino-1,2,4-triazol-1-yl)-D-alanine. Two other metabolites were found, but not identified. The same metabolites were present in rats. After treatment of the soil surrounding plants only small amounts of aminotriazole and its metabolites were translocated to the plant. In apples residues of the parent compound and the metabolite triazolylalanine were undetectable or very low: when present the compounds were in both free and conjugated forms. In cell suspension cultures from apples 3,5-dihydroxy-1,2,4-triazole was produced.

In soil rapid degradation occurs with CO_2 as the main degradation product. Degradation in soil is strongly influenced by the presence of micro-organisms, and does not occur under anaerobic conditions. From laboratory experiments it was possible to propose a degradation scheme for amitrole in soil. The ring is opened after metabolism to 5-hydroxyaminotriazole, and via cyanamide the compound is decomposed to CO_2 and ammonia. Because of the rapid degradation only small amounts of aminotriazole are leached into soil. Leaching is most pronounced in sandy soil with a low content of organic material.

New analytical methods for the determination of residues of amitrole have been developed using gas chromatography with a nitrogen-specific detector, thin layer chromatography, and high performance liquid chromatography with fluorescence or electrochemical detection. The limits of determination are 0.01-0.02 mg/kg for residues in fruit, vegetables and soil.

A complete re-evaluation of amitrole has not been possible because new data from supervised trials were not available. Although the registered uses reported to the Meeting are similar to the application conditions in some supervised trials examined by the JMPR in 1974, the data from the trials currently in progress should be taken into consideration. No reports from studies of storage stability were available, but the Meeting was informed that the results of such studies will be available in 1995. Reports of animal transfer studies were also lacking, but as residues of amitrole in crops are obviously very low and usually below the limit of determination, there is a very limited need for such studies.

RECOMMENDATIONS

No residue limits have been established for amitrole in food commodities but a note states that uses of amitrole should be restricted to those where residues in food would not be expected to occur. The Meeting recommends the addition to this note of the statement: "A realistic limit of determination for the general monitoring of amitrole would be 0.05 mg/kg."

FURTHER WORK OR INFORMATION

Desirable

1. Residue data from supervised trials on apples, pears and grapes known to be in progress.

2. Reports from experiments on the storage stability of amitrole known to be in progress.

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