GUAZATINE (114)

EXPLANATION

Guazatine, originally evaluated by the JMPR in 1978 and 1980, is included in the CCPR periodic review programme (ALINORM 91/24A, para 321 and Appendix VI, para 18). At the 1992 CCPR the manufacturer provided a list of all available data and indicated that toxicological studies were under way. Guazatine was tentatively scheduled for the 1996 JMPR pending the availability of these studies (ALINORM 93/24, Appendix V, Annex I). The 1995 CCPR postponed the residue review to 1997 (ALINORM 95/24A, Appendix IV). Information on current GAP was requested by circular letters (CL 1991/15-PR and CL 1993/11-PR).

The manufacturer provided data on physical and chemical properties, metabolism in plants and animals, environmental fate, methods of residue analysis, registered uses, residues in cereals, citrus fruits and sugar cane, and national MRLs (Buys *et al.*, 1997). Information on GAP and national MRLs was supplied by Australia (Anon., 1996a) and Germany (Anon., 1996b), and on GAP by Norway (Anon., 1997a) and the UK (Anon., 1997c). The Netherlands provided information on analytical methods, use patterns and national MRLs (Anon., 1997b).

IDENTITY

ISO common name: Guazatine

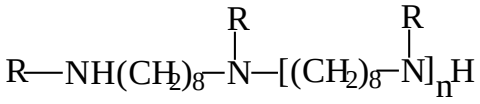
Chemical name:"A mixture of the reaction products from polyamines,
comprising mainly octamethylenediamine,
iminodi(octamethylene)diamine,
octamethylenebis(imino-octamethylene)diamine,
and carbamonitrile" (IUPAC)

The approved common name guazatine was originally defined as applying to 1,1'iminodi(octamethylene)diguanidine (BSI used the name *guanoctine* from 1970-1972). It is now known that the material marketed commercially is a reaction mixture. Produced by the amidination of technical iminodi(octamethylene)diamine, commercial guazatine containes numerous guanidines, in which the amino and imino groups of the polyamine chain form part, and polyamines; many of these bases are fungicidal. A replacement common name, iminoctadine (*q.v.*) has been established for 1,1'-iminodi(octamethylene)diguanidine. (Pesticide Manual, 1994).

CAS No: [115044-19-4] for guazatine acetates

Synonyms: GTA

Structural formula:



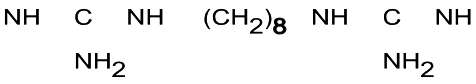
N may be 0,1,2 etc. and any R substituent may be
-H (17-23%) or
-$C(NH_2)=NH$ (77-83%).

A coding system is used for the compounds that make up guazatine in which "N" represents any amino group thus:

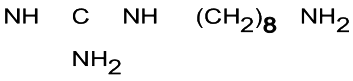
NN represents $H_2N-(CH_2)_8-NH_2$
NNN represents $H_2N-(CH_2)_8-NH-(CH_2)_8-NH_2$ and so on.

"G" represents any guanidated amino group (NH or NH_2) for example:

GG means



and GN means



The guanidated diamines and triamines are the most abundant components of guazatine. A typical composition of free guazatine (not of guazatine acetates, the salts which are used in practice) is as follows.

Component	%	Component	%
NN	0.8	GGG	30.6
GN	9.8	GNNG	1.4
GG	29.5	GGGN	1.4
NNN	≤0.1	GGGG	5.1
NGN	0.8	Other tetramines[1]	3.1
GNN	1.7	GGGGG	1.1
GGN	8.1	Other pentamines[1]	1.4
GNG	4.5	Hexamines and above[1]	0.6
		Total:	99.9

[1]And their guandidated derivates

It can be seen that diamine derivatives account for 40% of the constituents, triamines for 46%, tetramines for 11% and other amine derivatives for 3%. The most abundant individual components are

the fully guanidated triamine (GGG, 30.6%) and the fully guanidated diamine (GG, 29.5%) followed by the monoguanidated diamine (GN, 9.8%) and a diguanidated triamine (GGN, 8.1%).

Molecular formula: not applicable for a mixture
Molecular weight: not applicable for a mixture

Physical and chemical properties

Active ingredient as acetates

Vapour pressure: less than 10^{-5} Pa at °C (Boden, 1992a)
Melting point: guazatine acetate begins to melt at 60°C

Octanol/water partition coefficient (Karlsson and Stensiö, 1984, 1988):

pH	Component	log P_{ow}
3	guazatine acetates (GTA)	-1.2
7		ND
10		-0.9
6.5	1-amino-8-guanidino-octane diacetate (NG)	-2.3
6.5	1,8-diguanidino-octane diacetate (GG)	-3.3, -4.8
6.5	bis(8-guanidino-octyl)amine triacetate (GNG)	-3.2
6.5	bis(8-guanidino-octyl)guanidine triacetate (GGG)	-4.8

Solubility (Carlsson, 1992):

Solvent	Solubility at 20°C, g/l
water	> 3000
dimethylformamide	approx. 500
N-methylpyrrolidone	approx. 100
ethanol	200
methanol	510
2-propanol	28
N-octanol	15
acetone	<0.1
dichloromethane	<0.1
ethyl acetate	<0.1
toluene	<0.1
N-hexane	<0.1

r.d. (specific gravity): 1.09 g/cm^3 at 20°C (Boden, 1992b)

Hydrolysis: Guazatine acetates taken from two technical batches were hydrolysed in buffered aqueous solution at pH 5, 7 and 9 at 25°C for 30 days according to EPA Guidelines, and five compounds were determined by an HPLC method. There was no significant hydrolysis of any of the five components (Boden, 1992c).

Photolysis: Samples of GTA 70 in buffered aqueous solutions were irradiated in a Rayonet photochemical reactor at 300 nm for 24 h (Erikson and Stensiö, 1987) with the following results.

pH	Concentration, mg/l		
	Stored in darkness	Irradiated	% loss by photodegradation
5	26	26,3	0
7	8	6,5	19
9	18	13,5	25

Technical material

Purity: Technical guazatine obtained in the production process is a 70% w/w solution of guazatine acetates in water known as GTA 70. This solution is the basis of formulations.

Melting range: not applicable

Stability: GTA 70 is stable for at least 2 years at ambient temperature (can be stored at temperatures between 0°C and 50°C) (Carlsson,1993).

Formulations

LS: Solution for seed treatment
FS: Flowable concentrate for seed treatment
SL: Soluble concentrate
TC: Technical material
WP: Wettable powder
WS: Water-dispersible powder for slurry treatment

All active ingredient contents of the formulations are expressed as guazatine acetate. For example, a 200 SL formulation contains 200 g/l of guazatine acetate or 133 g/l of guazatine. The following products are used.

Post-harvest treatment of citrus fruit

SL 40 g/l guazatine acetate
SL 150 g/l guazatine acetate

SL 200 g/l guazatine acetate
SL 400 g/l guazatine acetate
TC 700 g/l guazatine acetate
Wax 3 g/l guazatine acetate

Seed treatment products

FS 150 g/l guazatine acetate, 12.5 g/l triticonazole
FS 266.7g/l guazatine acetate, 16.7 g/l triticonazole
FS 400 g/l guazatine acetate, 10 g/l flutriafol
LS 25 g/l guazatine acetate, 25 g/l imazalil
LS 150 g/l guazatine acetate, 10 g/l imazalil
LS 200 g/l guazatine acetate, 30 g/l imazalil
LS 200 g/l guazatine acetate, 200 g/l fenfuram, 20g/l imazalil
LS 200 g/l guazatine acetate, 12.5g/l triticonazole, 125g/l fipronil
LS 265 g/l guazatine acetate
LS 300 g/l guazatine acetate
LS 300 g/l guazatine acetate, 2.5 g/l cyproconazole
LS 300 g/l guazatine acetate, 5 g/l propiconazole
LS 300 g/l guazatine acetate, 15 g/l tebuconazole
LS 300 g/l guazatine acetate, 20 g/l imazalil
LS 300 g/l guazatine acetate, 25 g/l imazalil
LS 300 g/l guazatine acetate, 25 g/l triticonazole
LS 300 g/l guazatine acetate, 150 g/l fenfuram
LS 300 g/l guazatine acetate, 100 g/l fenfuram
LS 300 g/l guazatine acetate, 150 g/l fenfuram, 40 g/l imazalil
LS 350 g/l guazatine acetate
LS 400 g/l guazatine acetate
LS 700 g/l guazatine acetate
SL 200 g/l guazatine acetate
SL 400 g/l guazatine acetate
WP250 g/kg guazatine acetate

METABOLISM AND ENVIRONMENTAL FATE

Animal metabolism

Absorption, distribution and excretion

Rats. In a preliminary study (Leegwater, 1975) a male Wistar rat received a single oral dose of an aqueous solution (pH 5) containing guazatine labelled with tritium (19.5 μCi) in the octyl moieties and ^{14}C (5.84 μCi) in the guanidino groups. By the end of the 72-hour collection period the total recoveries of radioactivity were 83% of the ^{14}C and 93% of the ^{3}H. Most of the administered dose was recovered from the faeces (about 64% of the ^{14}C, 39% of the ^{3}H) and urine (about 15% ^{14}C, 42% ^{3}H). Most of the

radiolabel in the urine was found during the first 24 hours whereas most of that in the faeces was found between 24 and 48 hours, but it was noted that the rat did not eat during the first 24 hours and this would be likely to reduce the intestinal transit time. Similar proportions of the ^{14}C dose were found in the liver (0.61%) and kidneys (0.67%) but the proportions of ^{3}H differed: liver 1.6%, kidney 0.17%. After 72 hours the gastrointestinal tract accounted for 0.47% of the ^{14}C and 1.3% of the ^{3}H, and the residual carcase for 1.9% of the ^{14}C and 9.3% of the ^{3}H.

In a later study (Leegwater, 1980) two male Wistar rats were dosed by oral gavage with [^{14}C]guazatine (pH 5.8) labelled in the guanidino groups at the nominal level of 10 mg/kg body weight. Samples were collected until 120 hours after dosing. The mean total recovery of radioactivity was 93% with about 60% in the urine and 30% in the faeces. Elimination in the urine was rapid, with 93% of the total recovered being eliminated in the first 24 hours. In the faeces about 52% of the total was found during the first 24 hours. After five days a mean total of about 2.5% of the administered dose remained in the body. The liver contained 0.6% of the administered radioactivity, the kidneys 0.08%, the blood 0.41%, the gastrointestinal tract 0.22% and the carcase 1.2%.

In a third study (Cameron *et al.*, 1989) four groups of five rats were dosed orally with [^{14}C]guazatine labelled in the octyl chains. The first group received a single dose of 20 mg/kg bw, two groups received single doses at 2 mg/kg bw (one for an ADME[1] investigation and the other for a bile elimination investigation), and the fourth group received daily doses of 2 mg/kg bw over a period of fourteen days. The mean recoveries of the total radioactive residue (TRR) were about 100% for all three single dose groups (during 96 hours), distributed as shown in Table 1.

[1] Absorption, distribution, metabolism and excretion

Table 1. Distribution of ^{14}C in rats (Cameron *et al.*, 1989).

Sample	^{14}C, % of dose					
	20 mg/kg		2 mg/kg ADME		2 mg/kg Bile[1]	
	Male	Female	Male	Female	Male	Female
Urine	6.9	7.5	6.3	6.06	7.16	4.87
Cage wash	0.55	0.63	0.75	0.75	0.26	3.35
Faeces	92.9	94.0	95.2	95.71	54.08	55.75
CO_2[1]	0.84	0.73	0.66	0.54	na	Na
Bile	Na	na	na	na	0.04	0.23
GI tract	Na	na	na	na	40.3	23.88
Tissue	0.91	1.1	0.74	0.89	na	Na
Carcase	0.4	0.42	0.12	0.15	3.49	11.12
Total	101.8	103.8	103.2	103.7	105.3	99.2

ADME: Absorption, distibution, metabolism, excretion
na: not analysed
[1]Collected for first 24 hours

The TRR in the tissues after 96 hours was low. The highest levels were found in the kidneys (low dose 0.06-0.2, high dose 0.7-3.03 mg guazatine equivalents/kg) and liver (low dose 0.07-0.38, high dose 1.14-5.1 mg/kg). The high recovery of ^{14}C from the faeces and the low levels in the urine, tissues and bile indicate that the [^{14}C]guazatine was poorly absorbed.

After fourteen daily administrations of [^{14}C]guazatine there was some evidence that radioactivity had accumulated to a slight extent in the liver (0.31-1.9 mg guazatine equivalents/kg), kidneys (0.09-1.2 mg/kg) and fat (0.01-0.17 mg/kg) but not in the plasma or carcase. These levels all decreased over a 14-day depuration period leaving mean levels of 0.14 mg/kg in the liver, 0.55 mg/kg in the kidneys, 0.05 mg/kg in the fat, <0.01 mg/kg in the plasma and 0.05 mg/kg in the carcase.

The most recent study (Kato *et al.*, 1985) was published in the literature. Male rats were administered [^{14}C]guazatine triacetate labelled in the guanidine groups ([G-^{14}C]) or at the terminal positions of the octamethylene groups ([M-^{14}C]) according to the schedule shown in Table 2.

Table 2. Dose schedules in studies of metabolism of guazatine in rats (Kato *et al.*, 1985).

Group	Type of study	Administration	Radiolabel	Dose, mg/kg bw	Specific activity, µCi/mg	No of rats
1	elimination/balance	oral	[G-^{14}C]	3	45.4	4
2[1]	elimination/balance	oral	[G-^{14}C]	30	14.3	4
3[1]	elimination/balance	intravenous	[G-^{14}C]	3	45.4	4
4	elimination/balance	intravenous	[M-^{14}C]	3	13.0	4
5	metabolism	intraperitoneal	[M-^{14}C]	15	13.0	4

Group	Type of study	Administration	Radiolabel	Dose, mg/kg bw	Specific activity, μCi/mg	No of rats
6	bile	intravenous	[G-^{14}C]	3	45.4	4
7	bile	intravenous	[M-^{14}C]	3	13.0	4

[1]Blood samples taken for pharmacokinetic analysis

The mean total recoveries of radioactivity after seven days were μ95% from both oral and intravenous doses and yielded bioavailability values of about 8% for the oral administration. About 90% of the administered dose was recovered from the faeces of both oral dose groups with the urine containing about 4.6% of the dose. In both these groups about 1.2% of the dose remained in the body after seven days. The recovery of radioactivity from the faeces was similar for the two intravenous dose groups at about 27% of the dose. Higher proportions were eliminated in the urine, about 56% of the guanidine label and 38% of the methylene label. In both intravenous groups biliary excretion was low with means of 0.6-1.3% of the dose recovered in the first 24 hours. Faecal elimination of both radiolabels was about 9.3%, indicating that it was not entirely accounted for by biliary excretion. Urinary excretion of the two radiolabels again differed, about 20% of the guanidine and 5% of the methylene label being eliminated. Whole-body autoradiography provided evidence that salivary and gastrointestinal secretions were playing a role in the faecal elimination of guazatine.

The data on blood pharmacokinetics indicated that the mean concentration after oral administration at 30 mg/kg was 0.13 ± 0.03 mg guazatine equivalents/kg, with a maximum about 10 minutes after dosing. The elimination half-life was calculated to be 26.7 ± 2.1 hours. Tissue concentrations varied with the dose route. The oral groups showed a dose proportionality and similar distribution profile, with the highest concentrations in the kidneys. The intravenous dose groups showed a difference in the distribution profiles from the two labels, although the kidneys of both groups contained the highest TRR.

Cows. A study by Cameron and Phillips (1986) designed to investigate the disposition of [^{14}C]guazatine in six lactating cows was in two phases. In phase 1 two cows were given single intraruminal doses of [^{14}C]guazatine, one at 0.1 mg/kg bw, a level consistent with normal exposure to residues in the feed, and the other at ten times his level. Excreta and milk were collected for the whole period of the experiment, and blood, expired air and saliva were sampled periodically to assess the plasma and excretion kinetics of the distribution. In phase 2, six cows received [^{14}C]guazatine by intraruminal administration twice daily for 10.5 days (total 21 doses). Three cows were dosed at 0.5 mg/kg bw and three at 0.05 mg/kg bw. All the milk was collected and blood was sampled repeatedly. Each cow was slaughtered at the peak ^{14}C level in the plasma for identification of the major tissue residues.

The mean recoveries of radioactivity from the faeces after the single doses of 1 and 0.1 mg/kg were both about 93% of the dose and the recovery from the urine during 168 hours reached about 1.6%, giving a mean total recovery of about 95%.

Plasma concentrations after the administration of 1 mg/kg were very low with a peak of about 0.003 mg guazatine equivalents/l at 12 hours, and those from the 0.1 mg/kg dose were below the limit

of determination (0.0012 mg/l). After repeated doses of 0.5 mg/kg bw the levels of activity rose steadily to reach a plateau at day 6 which was maintained throughout the remainder of the dosing regime. The plateau levels in individual animals ranged from 0.007 to 0.014 mg/l. The levels in the animals dosed at 0.05 mg/kg bw were below the limit of reliable determination (<0.002 mg/l).

Excretion into the milk after single applications was minimal with levels below the limit of determination (0.0012 mg/l) from the low dose and a maximum recovery of about 0.02% of the dose after 24 hours from the high dose. After repeated dosing at 0.5 mg/kg the ^{14}C in the milk reached a plateau between 0.012 and 0.028 mg/l (in three cows) at 48-56 hours. After repeated dosing at 0.05 mg/kg residues were extremely low and appeared to reach a plateau by 48 hours between 0.0011 and 0.0038 mg/l. The highest proportion of the TRR found in the milk was in the whey fraction (a mean for all six animals of 58%), with 30% in the curd and 12% in the fat.

The liver and kidneys of the high-dose animals were found to contain the highest levels of absorbed radioactivity with respective means of 0.084 and 0.082 mg guazatine equivalents/kg. Muscle and fat contained negligible levels (<0.02 mg/kg).

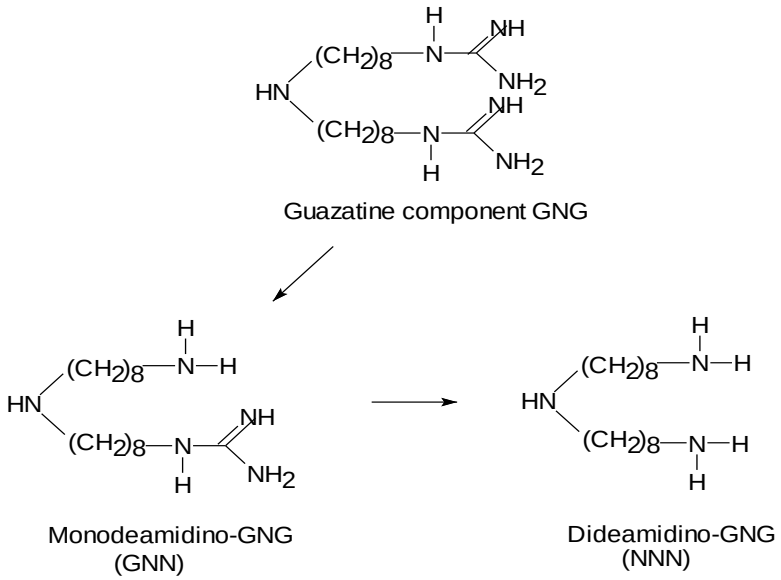
Biotransformation

Rats. In a preliminary study by Leegwater (1975) the differing ratios of $^{14}C/^{3}H$ in the various samples implied that some biotransformation of the administered guazatine had taken place but TLC of urine and faecal extracts did not produce any conclusive evidence of this. In the later study (Leegwater, 1980) TLC indicated the presence of one major and four minor radiolabelled components in the 0-24 hour urine which also appeared to be present in the administered guazatine preparation. A similar result was obtained with faecal extracts.

A review of the results obtained by Cameron *et al.* (1989) was recently reported (Prout, 1996). The results showed that up to 94% of an oral dose of [^{14}C]guazatine had been eliminated as the parent material within 96 hours in the faeces, with 6-7% of the dose being absorbed but largely eliminated in the urine in the same time. The biliary route appeared to play no, or very little, part in the elimination of absorbed material. The absorbed radioactivity appeared to be largely associated with two components of guazatine, G and GGG. The residues in the tissues support the biokinetic evidence for concluding that guazatine is poorly absorbed after oral administration. The metabolism of guazatine components in the rat, irrespective of the number of guanidino groups, mainly involved deamidination to the corresponding amino compounds which were largely excreted in the urine.

The most recent investigation (Kato *et al.*, 1985) indicated the presence of 16 components of which two were major. The major residues identified in the kidney extracts were guazatine, 1,1'-iminodi(octamethylene)-8-amino-8'-guanidine and 1,1'-iminodi(octamethylene)diamine. In the faecal extracts guazatine was the major component with monodeamidinoguazatine also identified. The metabolites in the urine were relatively hydrophilic. Thirteen components were detected but only monodeamidino-guazatine was identified. A general view of the metabolism is shown in Figure 1.

Figure 1. Proposed general metabolic pathway of guazatine components in mammals, using component GNG as an example (Kato *et al.*, 1985).



In general the results of this study are consistent with those obtained in the preceding studies on rats. The deamidination of a component of guazatine was shown to be one of the main routes of biotransformation in rats before the amines are largely excreted in the urine. The metabolic pathway is similar for the other guanidated components of guazatine (GG, GN, GGG, GGN).

Cows. Cameron *et al.* (1984) investigated the degradation of octyl- and guanidine-labelled [^{14}C]guazatine in a rumen fermentation system *in vitro* and a liver homogenate system. No significant degradation of either labelled compound was noted during incubation with fresh rumen contents although some radioactivity was recovered in the traps for gaseous products: 1.4% of the original guanidine radioactivity and 0.067% of the octyl. Aerobic incubation with fresh bovine liver homogenates however produced about 40% loss of radioactivity from the incubation mixture containing [*guanidine*-^{14}C]guazatine and about 10% loss of the octyl label. These losses were not accounted for by the radioactivity in the trapped gases which only accounted for 1.9% and 0.4% of the guanidine and octyl labels respectively. Analyses by TLC did not reveal any significant qualitative differences from the test materials.

In the study by Cameron and Phillips (1986) the radioactivity in the faeces (93% of the dose) and liver had similar chromatographic properties to the test material. The urine was found to contain polar component(s) with a chromatographic profile similar to that from the kidneys. Definitive identifications were not possible.

Plant Metabolism

The uptake, translocation and metabolism of [^{14}C]guazatine labelled at the terminal carbons of the octyl chains was investigated in winter wheat (Caley *et al.*, 1990a). The compound was applied as a seed dressing formulation at a level of 1.05 g/kg seed. Samples were collected about 5 weeks after emergence and again at maturity.

The uptake of guazatine from the seeds was low. At the first harvest the mean uptake into the plants was 0.07 % of the applied radioactivity, equivalent to 0.18 mg/kg as guazatine (Table 3). At maturity the ^{14}C in the plants still represented 0.07 % of the applied radioactivity but because of dilution by growth the concentrations in the plants from the treated and control plots were now similar and all mean values were less than 0.05 mg guazatine equivalents/kg (Table 4). As most of the residues at the final harvest were below the limit of reliable determination (0.01 mg guazatine equivalents/kg), the small differences observed between individual components and between test and control plots are not considered to be significant, but the mean concentrations of radioactivity in the grain were consistently lower than those in the straw and chaff. The levels of radioactivity in the soil samples were very low, almost all below the limit of reliable determination. The levels were too low to determine the nature of the residues or the extent of metabolism.

Table 3. ^{14}C in wheat plants 5 weeks after emergence, following application of [^{14}C]guazatine as a seed dressing (Caley *et al.*, 1990a).

^{14}C, mg/kg as guazatine equivalents, in plants (fresh weight)			
Plant no.	Plot 1	Plot 2	Plot 3
2	0.04	0.12	0.02
3	0.07	0.13	0.02
4	0.01	0.12	<0.01
5	0.03	0.16	<0.01
6	0.03	0.16	<0.01
7	0.05	0.76[1]	<0.01
8	<0.01	0.19	<0.01
9	0.01	0.17	<0.01
10	0.01	0.35	<0.01
Mean	0.03	0.18	0.01

Plots 1 and 3 untreated; plot 2 treated
[1]Outlier

Table 4. ^{14}C in wheat plants at final harvest following application of [^{14}C]guazatine as seed dressing (Caley *et al.*, 1990a).

Plant no.	^{14}C, mg/kg as guazatine equivalents								
	Plot 1			Plot 2			Plot 3		
	Straw	Chaff	Grain	Straw	Chaff	Grain	Straw	Chaff	Grain
1	0.04	0.09	<0.01	0.025	0.02	<0.01	<0.01	0.01	<0.01
2	0.01	0.06	0.01	0.03	0.02	<0.01	0.01	0.02	<0.01
3	0.04	0.03	0.01	0.01	0.01	<0.01	0.01	0.01	<0.01
4	0.02	0.01	<0.01	0.01	0.02	<0.01	<0.01	0.01	<0.01
5	<0.01	0.04	0.01	0.02	0.01	<0.01	0.01	0.02	<0.01
6	0.01	0.04	<0.01	0.03	0.02	<0.01	0.02	0.02	0.01

Plant no.	^{14}C, mg/kg as guazatine equivalents								
	Plot 1			Plot 2			Plot 3		
	Straw	Chaff	Grain	Straw	Chaff	Grain	Straw	Chaff	Grain
7	0.02	0.05	<0.01	0.02	0.01	<0.01	0.02	0.02	0.01
8	0.02	0.03	<0.01	0.03	0.09	<0.01	0.01	0.02	0.01
9	<0.01	0.01	<0.01	0.01	0.02	<0.01	0.02	0.02	<0.01
10	0.01	0.04	0.01	0.02	0.01	<0.01	0.02	0.03	0.01
Mean	0.02	0.04	0.01	0.02	0.02	<0.01	0.01	0.02	<0.01

Plots 1 and 3 untreated; plot 2 treated

The foliar application of [^{14}C]guazatine at a rate of 1.1 kg ai/ha to wheat plants 11 weeks before harvest resulted in mean ^{14}C residues equivalent to 29 mg guazatine/kg in the straw and 18 mg/kg in the chaff. The levels in the grain were significantly lower at to 0.8 mg/kg, equivalent to 1.3% of the total radioactivity present at maturity (Caley *et al.*,1990b).

In a laboratory study by Lowden *et al.* (1996), a mixture of GG, GN and GGG were applied to seed surfaces, and the seeds were planted in soil in metabolism vessels. Most of the seeds germinated; it was possible to distinguish the seeds from the soil and extract the seeds separately up to 29 days after planting. Analysis of these extracts indicated a change in the profile of components present on the seed with levels of the three compounds generally decreasing. There was a concomitant generation of $^{14}CO_2$ (Table 5; see also "Environmental fate in soil" below).

Table 5. Levels of GG, GGG and GN in or on treated wheat seed (Lowden *et al.*, 1996).

	^{14}C, % of applied							
	Loamy sand		Sandy loam		Low organic loamy sand		Clay loam	
Day 0	Unit							
	1	2	36	37	72	106	107	
GG	13.2	15.4	21.2	9.82	9.79	16.6	18.1	
GN	3.36	6.64	4.62	2.02	0	1.77	6.62	
GGG	9.4	13.1	6.92	7.01	15.1	14.9	2.64	
Day 7	Unit							
	11	14	40	54	80	82	110	120
GG	13.7	15.6	16.1	26.6	15.85	4.41	12.8	29.8
GN	5.57	1.64	2.0	0	28.0	0	7.65	9.4
GGG	3.7	0	3.05	21.7	12.2	0	0	3.81
Day 15	Unit							
	4	5	48	55	79	95	115	116
GG	12.1	7.01	3.7	5.56	8.07	9.77	3.91	6.65
GN	3.21	1.43	0.25	0.78	31.1	1.31	0.96	0

	^{14}C, % of applied							
	Loamy sand		Sandy loam		Low organic loamy sand		Clay loam	
GGG	9.55	5.26	0.88	8.6	2.97	1.76	0.7	1.18
Day 29	Unit							
	19	20	49	50	85	99	118	121
GG	3.38	0.6	10.1	0	6.27	0	0	0
GN	1.24	0	0.3	0	1.5	0	0	0
GGG	2.69	7.46	5.15	0.01	0	0	0	0

The penetration, translocation and metabolism of [^{14}C]guazatine applied to dwarf apple trees were determined over a period of 12 weeks under laboratory conditions (Sato *et al.*, 1985). When the guazatine was applied to the leaf surface or the fruit by brushing with 0.05 or 0.1 kg ai/hl its translocation during 12 weeks was extremely limited. Autoradiography showed no observable movement in the leaves, and in the fruit the radiolabelled material was principally retained on the surface. Quantitative determination of the TRR in treated leaves indicated a slow disappearance (half-life 67 weeks). After 12 weeks approximately 87% of the applied [^{14}C]guazatine was recovered, and only 21% had penetrated the leaf tissues. The results are given in Table 6.

Table 6. Distribution of radioactivity in fractions from apple leaves (Sato *et al.*,1985).

Fraction	Identity	^{14}C, % of applied, at weeks after treatment					
		0	1	2	4	8	12
Surface washings		93.5	85.6	86.2	84.5	75.5	66.0
	Guazatine	na	67.9	67.0	64.2	61.9	52.8
	PM	na	7.4	9.0	10.3	7.7	6.3
	Others	na	10.2	10.2	10.0	5.9	6.9
Acetic methanol extracts		1.7	4.8	4.0	4.2	5.9	7.2
	Guazatine	na	2.5	1.3	1.1	2.6	1.7
	PM	na	0.3	0.4	0.4	0.7	0.8
	Others	na	2.0	1.8	2.7	2.6	4.6
Methanolic NaOH extracts		3.6	6.0	6.2	6.5	9.0	10.3
Aqueous		0.1	1.0	1.2	1.4	2.2	2.8
$CHCl_3$		3.5	5.0	5.0	5.1	6.8	7.5
	Guazatine	na	4.4	4.6	4.4	5.7	6.5
	PM	na	0.1	0.1	0.3	0.3	0.2
	Others	na	0.5	0.2	0.4	0.8	0.8
Unextractable residues		0.3	1.4	2.2	2.5	2.6	3.8
Total		99.2	97.9	98.6	97.7	93.0	87.3

PM: major photodegradation product
na: not analysed

Table 7. Distribution of radioactivity in fractions from apple fruits, 12 weeks after treatment (Sato *et al.*,1985).

Fraction	Identity	^{14}C, %
Surface washings		61.8
	Guazatine	56.6
	PM	2.9
	Others	2.7
Acetic acid methanol exctracts		9.4
	Guazatine	5.7
	PM	1.0
	Others	2.7
Methanolic NaOH extracts		23.6
Aqueous phase		3.0
$CHCl_3$ phase		20.6
	Guazatine	18.7
	PM	0.6
	Others	1.3
Unextractable residues		5.2

PM: major photodegradation product
Each value is the mean of duplicate experiments

Quantitative determination of the TRR in the fruits also confirmed the results of the autoradiography. Table 7 shows that 81% of the TRR in or on fruits analysed 12 weeks after treatment was still the parent mixture. The remainder comprised a major photodegradation product (4.5%), other extractable compounds (6.7%), and unextractable residues (5.2%). A similar distribution (61% of the residues as the parent mixture) was seen in leaves (Table 6).

The availability of guazatine residues in the soil to soya bean and rice plants was investigated under laboratory conditions with three Japanese soils (Kumagaya clay loam and Chiba loam under upland conditions, Nagaoka clay loam under flooded conditions) which were treated with 5 mg/kg of [^{14}C]guazatine triacetate (dry weight basis); some samples were kept for 26 weeks before planting to produce aged soil residues (Sato *et al.*, 1984).

The ^{14}C was determined in separate parts of soya bean plants grown in the Kumagaya and Chiba upland soils. Four weeks after planting, the TRR expressed on a dry weight basis in each part of the plants was less than one-fifth of that in the surrounding soil. The total recoveries of ^{14}C from the foliage and whole plants in the Kumagaya soil were only 0.08 and 0.13% of the applied radioactivity respectively (Table 8).

Table 8. Uptake of ^{14}C from upland soils by soya bean plants (Sato *et al*., 1984).

Sample	Kumagaya soil Weeks after planting 2	4	9	Chiba soil Weeks after planting 2	4	9
^{14}C as guazatine equivalents, mg/kg dry weight						
Stalk	0.036	0.478	-	0.034	0.473	-
First true leaves	0.035	0.259	-	0.030	0.229	-
Second true leaves	0.033	0.337	-	0.034	0.291	-
Third true leaves	0.038	0.77	-	0.035	0.392	-
Fourth true leaves	0.039	0.605	-	0.038	0.621	-
Developing leaves	-	0.329	-	-	0.443	-
Root	0.770	0.909	-	0.127	0.587	-
Pods	-	-	0.052	-	-	0.076
Seeds	-	-	0.053	-	-	0.084
% of applied ^{14}C						
Foliage	0.004	0.077	-	0.003	0.116	-
Whole plant	0.026	0.125	-	0.010	0.179	-

The residues in flooded Nagaoka soil were also not available to rice plants, which absorbed only 0.13% of the ^{14}C applied to the soil during a period of 4 weeks when the residues had been aged for 26 weeks (Table 9).

Table 9. Uptake of aged and freshly deposited [^{14}C]guazatine residues from flooded soils by rice plants (Sato *et al*., 1984).

Time after planting (weeks)	Residues aged 1 hour 1	2	4	Residues aged 26 weeks 1	2	4
^{14}C as guazatine equivalents, mg/kg dry weight						
Shoot	15.4	5.74	2.37	0.84	0.48	0.23
Root	19.6	6.61	2.36	0.91	0.55	0.34
% of applied ^{14}C						
Shoot	0.52	0.76	0.67	0.04	0.07	0.05
Whole plant	0.98	1.27	1.17	0.07	0.11	0.13

When the roots were treated with a nutrient solution containing 5 mg/kg of [^{14}C]guazatine they absorbed the extremely high concentration of ^{14}C of 2700 mg/kg in days, but there was little translocation into the shoots which contained only 7 mg/kg after 7 days.

Environmental fate in soil

Degradation

In a laboratory study (Lowden *et al.*, 1996) a mixture of radiolabelled GGG, GG and GN, the three main components of guazatine, was applied to wheat seeds at the commercial rate of 0.6 g ai/kg seed and the seeds were planted in four soils. The treatment rate is equivalent to an application to the soil of about 0.12 kg ai/ha at a sowing rate of 200 kg of wheat seeds per hectare. The seeds were planted in flasks containing 100 g (oven-dried equivalent) of soil, one seed per flask. The soils were a loamy sand, a sandy loam, a low organic matter loamy sand and a clay loam. The three compounds all decreased steadily with time. As early as day 7 significant quantities of $^{14}CO_2$ (4-14% of the applied radioactivity) were observed: similar behaviour of the compounds was observed in a leaching study (McMillan-Staff and Austin, 1996). The decrease of the initially-applied compounds was accompanied by the appearance of many minor degradation products, at low levels at all times (<0.05 mg guazatine equivalents/kg). These could be chromatographically characterized as diamines and monoamines, but were not positively identified owing to their very low concentrations. The deguanidated products GN and GNG have been confirmed in soil extracts. The results show that degradation occurs by two routes: deguanidation and oxidation of the octyl chains. This is consistent with a previous Swedish study on guazatine (Björk and Siirala-Hansen, 1986) which postulated deguanidation followed by oxidative degradation of the hydrocarbon groups. The other components in the commercial guazatine mixture would be expected to show a similar pattern of degradation. The components of technical guazatine not represented in this study (about 30%) comprise about 15% triamines, 11% tetramines and 4% pentamines or higher amines. The study has shown rapid degradation of the fully guanidated triamine and the other triamines would be expected to show similar or more rapid degradation. There is no reason to believe that the mechanisms of degradation of the higher oligomers would be significantly different since the components are chemically very similar to those studied. Figure 2 shows the proposed metabolic pathways using the fully guanidated triamine, GGG, as an example. For the sake of clarity the deguanidation reactions have been shown as occurring before the oxidations. In compounds that are only partially guanidated (either originally or as a result of the degradative process) the reactions are likely to be concomitant (Björk and Siirala-Hansen, 1986).

Half-lives of the components of guazatine in this study have been calculated as 62 days in loamy sand, 104 days in clay loam, 106 days in loamy sand with low organic matter, and 176 days in sandy loam. However, these were based on the total radioactivity unextractable by KOH reflux plus the CO_2 generated and so represent an extreme case. It should also be noted that the seeds were not allowed to develop far beyond germination and under field conditions, where the plants become established, degradation might be expected to be more rapid.

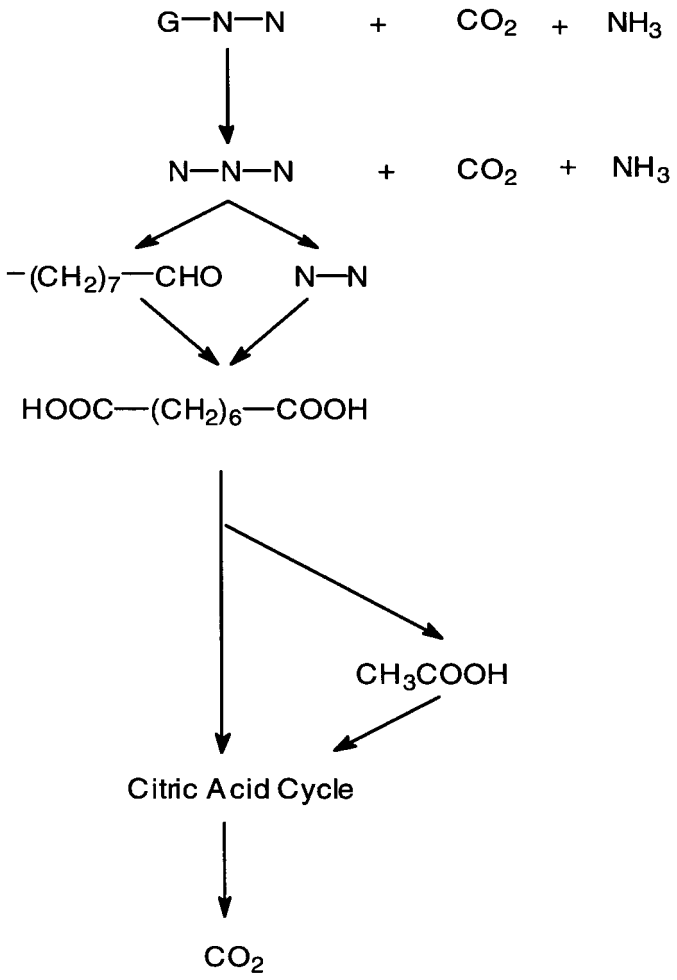
These results are also consistent with the previously mentioned study by Björk and Siirala-Hansen (1986). The half-life of guazatine dressed on to wheat seed at 0.75 g/kg seed was 20 days when the seed was incubated in Petri dishes at 20□C, and 80 days when the seed was sown in pots of soil stored outside. The data demonstrate a substantial influence of the test system on the rate of degradation of guanidated amine acetates.

Leaching

A study by McMillan-Staff and Austin (1996) was designed to investigate the mobility of three of the main compounds of guazatine when applied to wheat seeds which were subsequently planted in the tops of columns of three different soils which were leached to simulate rainfall. The compounds were

labelled with ^{14}C and two seeds were planted in the top of each column. After the equivalent of 50 cm of rain the guazatine components were still associated with the seeds or the soil surrounding them. The compounds that had moved from the seeds to the soil were not leached as only very small amounts of radiolabelled material were found either in the soil below that in which the seeds had been planted or in the leachate from the columns. During the leaching period there was significant mineralization of these compounds to carbon dioxide, showing that they are readily degraded in soil (probably at the seed surface).

Figure 2. Proposed pathways of degradation of guazatine components in soil, exemplified by the fully guanidated triamine (GGG).



The major components of guazatine (GG, GN and GGG) have been shown to be immobile in a range of soil types under conditions of simulated rainfall, while being mineralized to carbon dioxide. It is reasonable to extrapolate the results to the other components of guazatine. They are all similar physico-chemically in that they are ionic and differ only in the number of hydrocarbon chains and the combination of guanidated and unguanidated amine groups, and so would be likely to show similar leaching behaviour and degradation. The results, therefore, indicate that no movement of guazatine to deeper soil layers would be expected and that the potential for groundwater contamination would be extremely low.

METHODS OF RESIDUE ANALYSIS

Analytical methods

Such a complex mixture as guazatine presents a problem in choosing a residue analytical method. It is not practical to attempt analysis for all the components so some alternative is necessary. Two approaches have been used: (1) "total residue" methods involving the generation of a single compound and (2) the use of a major component as a "marker", with the inclusion of a correction factor to give the total residue (GG, representing 30% of the total free amine content of the product, is the marker of choice. All the residues are expressed as guazatine.

Citrus fruits. In a total residue method (Thornberg, 1979a; Thorstensson and Stensiö, 1984; Stensiö and Thorstensson, 1990) the guanidines are extracted as ion-pairs with picric acid into butanol, then extracted with sulfuric acid and hydrolysed to the parent amines. The amines are extracted into benzene and converted to trifluoroacetylated bis(8-amino-octyl)amine, which is determined by GC-MS. The method was valided by Stensiö (1986). The limits of determination for fortified orange pulp, wet peel and dried peel were 0.05, 0.2 and 1 mg/kg respectively. Table 10 shows the results of recovery studies.

Table 10. Recoveries of guazatine from fortified orange products (Stensiö, 1986).

Sample	Fortification, mg/kg	Recovery, %	Relative SD, %
Finisher pulp	0.05	128	11
	0.1	118	7
	0.2	119	7
Wet peel	0.2	113	20
	0.4	96	24
	0.8	110	5
Dried peel	1	138	12
	2	113	10
	4	111	12

Sugar cane. The method described above for citrus fruits was used by Thornberg (1979b) to determine guazatine in sugar cane. It was stated in the report that the limit of detection was 0.1 mg/kg but no validation of the method was reported.

Cereals. A method based on the aqueous acid or methanolic alkaline extraction of the active ingredient from the crop, followed by alkaline hydrolysis to bis(8-amino-octyl)amine was reported (Anon., 1974). The triamine was extracted into butanol and determined by GLC with a flame ionisation or nitrogen-selective detector. The recovery from oats fortified with guazatine at 1 mg/kg averaged 63% and ranged from 56 to 71% (7 values).

A residue method has been applied to rice by Kobayashi *et al.* (1977). After extraction with alkaline methanol, clean-up by liquid-liquid partition and treatment with hexafluoroacetyl acetone, the residues were determined by GLC with an AFID in the nitrogen mode as bis[3,5-bis(trifluoromethyl)pyrimidyl-1-amino]-8,8´-dioctylamine. The limit of determination was 0.05 mg/kg and recoveries ranged from 80 to 99%.

Guazatine was extracted as an ion-pair with picric acid from cereal grains into butanol (Thornberg, 1976a). After extraction with sulfuric acid, the guazatine was hydrolysed to the triamine which was extracted into benzene and, after trifluoroacetylation, determined by GC-MS. A detection limit of 0.1 mg/kg was reported but the recovery was only 10-20%.

In a later method (Thornberg, 1979c) grain or straw samples were extracted with hydrochloric acid and the guanidines extracted as ion-pairs with picric acid from the aqueous phase into butanol. After extraction with sulfuric acid the guanidines were hydrolysed to the parent amines. The amines were extracted into benzene and converted to the trifluoroacetylated derivative, *N*,*N*-bis(8-trifluoroacetamido-octyl)trifluoroacetamide, which was determined by GC-MS using multiple ion detection. A limit of detection of 0.05 mg/kg and a recovery of about 50% were claimed in the text but no validation was reported.

This method was improved by Risholm-Sundman *et al.* (1988) by the addition of an internal standard before the extraction. The limit of determination was stated to be 0.05 mg/kg but the lowest fortification level was 0.5 mg/kg. The recoveries from grain were 68-118 % and from straw 100-116 % after fortification with 0.5, 3 and 10 mg/kg.

A new method was developed by Stensiö (1990). The guanidino compounds of guazatine are all extracted, but the purification and derivatization steps are designed for the determination of only one of the major components, 1,8-diguanidino-octane (GG). After addition of internal standard (1,6-diguanidinohexane), guazatine is extracted from the sample with hydrochloric acid. Part of the extract is diluted with water and a 25% ammoniacal solution is added. Purification is carried out on a cation exchange column which is eluted with a 1:1 mixture of water and glacial acetic acid. Guazatine and the internal standard are derivatized in pyridine with hexafluoroacetylacetone, and the derivatives are cleaned up on an alumina column. The derivative of 1,8-diguanidino-octane is determined by GC-MS with internal standard calibration. The limit of determination is 0.05 mg/kg guazatine. The relative standard deviations calculated from 28 analyses of spiked wheat grain samples ranged from 2.9 to 20% in the concentration range 0.05-1 mg/kg guazatine, with recoveries of 64 to 82%.

This performance was maintained in more recent work (Fuchsbichler, 1992a,b) carried out with the compound GG and determination as the same derivative, again by GC-MS with internal standard calibration. The recoveries from cereal ears, straw and grain are shown in Table 11.

Table 11. Recoveries of GG from cereal fractions (Fuchsbichler, 1992a,b).

Sample	GG fortification, mg/kg	Recovery, %
Ears	0.2	82 (mean of 2)
Ears	0.1	97 (mean of 2)
Ears	0.05	96 (mean of 2)
Straw	1.0	82
Straw	0.1	78
Grain	0.2	110 (mean of 4)
Grain	0.05	97 (mean of 4)

Stability of residues in stored analytical samples

The stability of residues of guazatine in cereals was studied by storing analysed wheat samples (straw, grain and ears) at -20ºC and reanalysing after 2 years (Risholm-Sundman and Jonsson,1989). The study was inadequate as the analytical method had not been validated.

Definition of the residue

The metabolism of guazatine has not been fully characterized in either animals or plants. The Meeting concluded that its residues in products of animal origin could not be defined.

The main uses of guazatine are for the seed treatment of cereals and post-harvest application to citrus fruits. The Meeting concluded that the available studies are adequate only to define the residue arising from the seed treatment of cereals. Should further uses be planned in future (e.g. foliar sprays or use on plants other than cereals), detailed metabolism studies would be required.

The determination of total guazatine residues is based on conversion to the triamine bis(8-amino-octyl)amine, which also occurs as a metabolite. Modern analytical methods using 1,8-diguanidino-octane (GG), one of the main components of guazatine, as a marker are more specific.

The Meeting recommended that the definition of the residue for enforcement purposes should be changed to "octane-1,8-diyldiguanidine", i.e. 1,8-diguanidino-octane, "GG". Assuming that the content of GG is 30% of the total free base content, a conversion factor of 3 is required for risk assessment purposes for commodities of plant origin.

Residue definition for enforcement purposes for cereal grains: octane-1,8-diyldiguanidine ("GG"), expressed as octane-1,8-diyldiguanidine.

Residue definition for risk assessment purposes for cereal grains: guazatine.

USE PATTERN

Guazatine is a non-systemic contact fungicide which disturbs the membrane function of fungi, decreasing the cellular permeability. The decrease in oxidative capacity is probably due to the inhibition of the uptake of certain substrates rather then a direct effect on enzymes. It acts at several sites, which prevents resistance.

Guazatine controls a wide range of seed-borne diseases of cereals, e.g. seedling blight *(fusarium spp.)*, glume blotch *(septoria)*, common bunt *(tilletia spp.)*, common root rot *(helminthosporium)* and smut *(ustilago)*. It is used on citrus fruits as a bulk dip after harvest, in the packing line as a spray and in washing installations to disinfect the process water. It controls sour rot *(geotrichum candidum)*, green mould *(penicillium digitatum)* and blue mould *(penicillium italicum)*.

The Meeting received information on GAP from the manufacturer and the governments of Australia (Anon., 1996a), Germany (Anon., 1996b), Norway (Anon., 1997a), The Netherlands (Anon.,

1997b) and the UK (Anon., 1997c). Tables 12-14 show the registered uses of guazatine for seed treatment, post-harvest application, and other uses. The application rates refer only to guazatine, although some formulations are mixed.

Table 12. Registered uses of guazatine for seed treatment. All single applications.

Crop	Country	Application	
		Form	Rate, a ai/kg seed
Wheat, rye, barley, oats, triticale	Austria	LS 350 g/l	0.875
		LS 300 g/l	0.6
	Belarussia	LS 300 g/l	0.45-6
	Belgium	LS 350 g/l	1.05
		LS 300 g/l	0.9
	Brazil	WP 250 g/kg	0.75
	Bulgaria	LS 300 g/l	0.6
	Croatia	LS 300 g/l	0.45-6
	Czech Republic	LS 350 g/l	0.7
		LS 300 g/l	0.45-0.6
	Denmark	LS 300 g/l	0.6
		LS 200 g/l	0.45
		LS 25 g/l	0.05
	Finland	LS 300 g/l	0.6
	France	LS 265 g/l	0.8
		FS 400 g/l	
	Germany	LS 350 g/l	0.7
		LS 300 g/l	0.6
		LS 200 g/l	0.4
		FS 300 g/l	0.6
		FS 150 g/l[1]	0.6
	Hungary	LS 350 g/l	0.7-1.05
		LS 300 g/l	0.6-0.9
	Italy	LS 300 g/l	0.6
		LS 325 g/l	
	Kahzahkstan	LS 300 g/l	0.45-0.6
	Macedonia	LS 350 g/l	0.7-1.05
	The Netherlands	LS 350 g/l	0.7
		LS 300 g/l	0.6-0.7
	Norway	LS 300 g/l	0.6
		LS 25 g/l	0.05
	Poland	LS 350 g/l	0.7-1.05
		LS 300 g/l	0.45- 0.6
	Romania	LS 350 g/l	0.7 -1.05
		LS 300 g/l	0.45- 0.6

Crop	Country	Application	
		Form	Rate, a ai/kg seed
	Russia	LS 350 g/l	0.7
		LS 300 g/l	0.45- 0.6
	South Africa	SL 400 g/l	0.8
	Spain	LS 300 g/l	0.6-0.9
	Sweden	LS 400 g/l	0.6
		LS 350 g/l	0.6
		LS 300 g/l	0.6
		LS 150 g/l	0.6
		LS 25 g/l	0.05
	UK	LS 300 g/l	0.6-0.9
	Ukraine	LS 350 g/l	0.7
		LS 300 g/l	0.45-0.6
	Uzbekistan	LS 350 g/l	0.7

[1]Pending

Table 13. Registered post-harvest uses of guazatine. All single applications.

Crop	Country	Form	Application	
			Method	Rate, kg ai/hl
Citrus	Argentina	SL 40 g/l	watering	0.2
	Australia	SL 400 g/l	watering	0.052
	Greece	SL 400 g/l	watering	0.16
	Morocco	SL 200 g/l	watering	0.1
	Spain	SL 200 g/l	watering	0.06-0.1
	South Africa	Wax 3 g/l[1]		0.3
		SL 200 g/l	dipping	0.1
	Uruguay	SL 40 g/l	watering	0.2
Rockmelons	Australia	SL 400 g/l	Dipping[2]	0.052
Tomatoes	Australia	SL 400 g/l	Spraying[3]	0.12

[1]Apply to freshly washed and dry citrus at a rate between 1.3 and 1.5 l per 1000 kg of fruit to obtain a residue of 3.9 to 4.5 mg/kg
[2]Dipping for 1 minute within 24 h of harvest
[3]Spraying for 30 seconds as soon as possible, not later than 24 h after harvest

Table 14. Other registered uses of guazatine.

	Country	Form	Application			PHI, days
			Method	Rate	Number	
Rape	Germany	SL 600 g/l	spraying	1.2 kg ai/ha	1	56

	Country	Form	Application			PHI, days
			Method	Rate	Number	
Sugar cane	South Africa	SL 400 g/l	treatment of plant segment before planting	0.08 kg ai/hl water	1	

RESIDUES RESULTING FROM SUPERVISED TRIALS

The results of supervised residue trials on cereals, sugar cane and citrus fruit are shown in Tables 15 to 17. There were no trials of spraying rape, for which there is German GAP, or on post-harvest applications to rockmelons and tomatoes (Australian GAP).

Underlined residues in the Tables reflect current GAP. Double underlined residues have been selected for the estimation of supervised trials median residue (STMR) levels. All residues are expressed as guazatine.

Cereals (Table 15). The results of 84 trials on barley (21), oats (12), rye (4) and wheat (47) were reported from field trials in Brazil (Risholm-Sundman, 1984, 1986), France (Müller, 1996a-g), Germany (Fuchsbichler, 1995, 1996; Thornberg, 1978; Jonsson and Risholm-Sundman, 1986, 1987, 1988; Strätz, 1994, 1996), Italy (Müller, 1996h,i), Sweden (Thornberg, 1977), South Africa (Thornberg, 1976b) and the UK (Anon., 1972, 1973). As the analytical methods used before 1990 were not satisfactory only the wheat trials in 1994 and 1995 could be used for evaluation.

Table 15. Residues of guazatine in cereals from seed treatments. All single applications.

Crop, Country, Year	Form	Application, g ai/kg seed	Sample	PHI, days	Residues, mg/kg	Report
Barley, Spring						
Germany, 1979	WP	0.9	grain straw	120 120	<0.05 <0.05	438249 R 15
Germany, 1978	LS	0.9	grain straw	153 153	<0.05 <0.05	438248 R 42
Germany, 1979	LS	0.6	grain straw	151 151	<0.05 <0.05	438249 R 13
Germany, 1979	LS	0.4	grain straw	120 120	<0.05 <0.05	438249 R 17
Sweden,	LS	0.6	grain	112	<0.1	438245

Crop, Country, Year	Form	Application, g ai/kg seed	Sample	PHI, days	Residues, mg/kg	Report
1974						E-län
Sweden, 1974	LS	0.6	grain	127	<0.1	438245 D-län
Sweden, 1974	LS	0.6	grain	144	<0.1	438245 C-län
UK, 1972	LS	0.8	grain	154	<0.1	RT/19/72
UK, 1972	WP	0.8	grain	154	<0.1	RT/25/72
UK, 1972	LS	0.8	grain	150	<0.1	RT/20/72
UK, 1972	WP	0.8	grain	150	<0.1	RT/26/72
UK, 1973	LS	0.8	grain	151	<0.1	RT/12/73
UK, 1973	LS	0.8	grain	135	<0.1	RT/13/73
UK, 1973	WP	0.6	grain	151	<0.1	RT/15/73
UK, 1973	WP	0.6	grain	135	<0.1	RT/14/73
UK, 1973	WS	0.6	grain	151	<0.1	RT/16/73
UK, 1973	WS	0.6	grain	135	<0.1	RT/17/73
UK, 1973	WS	0.6	grain	151	<0.1	RT/19/73
UK, 1973	WS	0.6	grain	135	<0.1	RT/18/73
Barley, Winter						
Germany 1977	LS	0.4	grain straw	287 287	<0.05 <0.05	438248 R 48

Crop, Country, Year	Form	Application, g ai/kg seed	Sample	PHI, days	Residues, mg/kg	Report
Germany 1977	LS	0.4	grain straw	258 258	<0.05 <0.05	438248 R 49
Oats						
Germany, 1978	LS	0.9	grain straw	123 123	<0.05 <0.05	438248 R 44
Germany, 1978	LS	0.9	grain straw	140 140	<0.05 <0.05	438248 R 170
Germany, 1978	LS	0.9	grain straw	145 145	<0.05 <0.05	438248 R 171
Germany, 1979	LS	0.6	grain straw	123 123	<0.05 <0.05	438249 R 12
Germany, 1985	LS	0.6	foliage grain straw	59 132 132	<0.05 <0.05 <0.05	86-AC-0322 Rs 8501 B3
Germany, 1985	LS	0.6	foliage grain straw	44 127 127	<0.05 <0.05 <0.05	86-AC-0322 Rs 8501 E2
Sweden, 1974	LS	1.0	grain	154	<0.1	438245
Sweden, 1974	LS	1.0	grain	178	<0.1	438245
UK, 1972	LS	0.8	grain	154	<0.1	RT/22/72
UK, 1972	WP	0.8	grain	154	<0.1	RT/24/72
UK, 1972	LS	0.8	grain	149	<0.1	RT/21/72

Crop, Country, Year	Form	Application, g ai/kg seed	Sample	PHI, days	Residues, mg/kg	Report
UK, 1972	WP	0.8	grain	149	<0.1	RT/27/72
Wheat						
Brazil, 1985	LS	1.5	grain	109	<0.05	86AC0325
Brazil, 1985	LS	1.5	grain	114	<0.05	86AC0325
Brazil, 1985	LS	1.5	grain	114	<0.05	86AC0326
Brazil, 1985	LS	1.5	grain	109	<0.05	86AC0326
South Africa[1], 1976	LS	0.4	grain		<0.1	438247
South Africa[1], 1976	LS	0.8	grain		<0.1	438247
South Africa[1], 1976	LS	1.2	grain		<0.1	438247
Wheat, Spring						
France, 1995	FS	0.8	grain straw	140 140	≤0.05 ≤0.1	RD/CRLD/ AN/9615878 95507BX1
France, 1995	FS	0.8	grain straw	128 128	≤0.05 ≤0.1	RD/CRLD/ AN/9615878 95507AM1
France, 1995	FS	0.8	grain straw	131 131	≤0.05 ≤0.1	RD/CRLD/ AN/9615878 95507RS1

Crop, Country, Year	Form	Application, g ai/kg seed	Sample	PHI, days	Residues, mg/kg	Report
France, 1995	FS	0.8	grain straw	145 145	≤0.05 ≤0.1	RD/CRLD/ AN/9615878 95507LY1
France, 1995	FS	0.8	shoot grain straw	25 47 74 131 131	0.17 <0.1 <0.1 ≤0.05 ≤0.1	RD/CRLD/ AN/9615911 95518RS1
Germany, 1979	LS	0.6	grain straw	117 117	<0.05 <0.05	438249 R 14
Germany, 1979	LS	0.4	grain straw	115 115	<0.05 <0.05	438249 R 18
Germany, 1979	WP	0.9	grain straw	120 120	<0.05 <0.05	438249 R 16
Germany, 1981	LS	0.6	grain straw	144 144	<0.05 <0.05	438249 R 46
Germany, 1981	LS	0.6	grain straw	156 156	<0.05 <0.05	438249 R 47
Germany, 1985	LS	0.6	foliage grain straw	81 162 162	<0.05 <0.05 <0.05	86-AC-0322 Rs 8501 B1
Germany, 1985	LS	0.6	foliage grain straw	59 140 140	<0.05 <0.05 <0.05	86-AC-0322 Rs 8501 E1
Germany, 1986	LS	0.6	foliage grain straw	32 125 125	<0.05 <0.05 <0.05	87-AC-0172 Rs 8602 B2
Germany, 1986	LS	0.6	foliage grain straw	80 167 167	<0.05 <0.05 <0.05	88-AC-0215 CGD 34-86R

Crop, Country, Year	Form	Application, g ai/kg seed	Sample	PHI, days	Residues, mg/kg	Report
Germany, 1986	LS	0.6	foliage grain straw	80 167 167	<0.05 <0.05 <0.05	88-AC-0215 CGD 35-86R
Germany, 1986	LS	0.6	foliage grain straw	72 144 144	<0.05 <0.05 <0.05	88-AC-0215 CGD 36-86R
Germany, 1987	LS	0.6	foliage grain straw	85 157 157	<0.05 <0.05 <0.05	88-AC-0215 CGD 36-87R
Germany, 1987	LS	0.6	foliage grain straw	84 176 176	<0.05 <0.05 <0.05	88-AC-0215 CGD 37-87R
Germany, 1987	LS	0.6	foliage grain straw	86 157 157	<0.05 <0.05 <0.05	88-AC-0215 CGD 38-87R
Germany, 1994	FS	0.6	grain straw	125 125	≤0.05 <0.1	R1/94 RPA 21083 RP 94-593
Germany, 1994	FS	0.6	grain straw	114 114	≤0.05 <0.1	R1/94 RPA 21084 RP 94-593
Germany, 1994	FS	0.6	grain straw	143 143	≤0.05 <0.1	R1/94 RPA 21085
Germany, 1994	FS	0.6	grain straw	105 105	≤0.05 0.1	R1/94 RPA 21086
Germany, 1995	FS	0.6	grain straw	122 122	≤0.05 <0.1	R2/95 RPA 21087 RP 95-682
Germany, 1995	FS	0.6	grain straw	150 150	≤0.05 <0.1	R2/95 RPA 21088

Crop, Country, Year	Form	Application, g ai/kg seed	Sample	PHI, days	Residues, mg/kg	Report
						RP 95-682
UK, 1972	LS	0.8	grain	150	<0.1	RT/23/72
UK, 1972	WP	0.8	grain	150	<0.1	RT/28/72
Wheat, Winter						
France, 1995	LS	0.8	shoot	20	0.14	RD/CRLD/ AN/9615860 95506OR1
				27	<0.1	
				39	<0.1	
				88	<0.1	
				159	<0.1	
			grain	265	≤0.05	
			straw	265	≤0.1	
France, 1995	LS	0.8	shoot	109	<0.1	RD/CRLD/ AN/9615860 95506AV1
				144	<0.1	
			grain	215	≤0.05	
			straw	215	≤0.1	
Germany, 1986	LS	0.6	foliage	212	<0.05	87-AC-0172 Rs 8532 E1
			grain	286	<0.05	
			straw	286	<0.05	
Italy, 1994	FS	0.6	grain	213	≤0.05	RD/CRLD/ AN/9516718
			straw	213	≤0.1	
Italy, 1994	FS	0.6	grain	245	≤0.05	RD/CRLD/ AN/9516716
			straw	245	≤0.1	
Italy, 1995	FS	0.6	grain	219	≤0.05	RD/CRLD/ AN/9615957 95610BO1
Italy, 1995	FS	0.6	grain	219	≤0.05	RD/CRLD/ AN/9615957 95610BO2
Italy, 1995	FS	0.6	grain	219	≤0.05	RD/CRLD/ AN/9615958 95609BO1
			straw	219	≤0.1	

Crop, Country, Year	Form	Application, g ai/kg seed	Sample	PHI, days	Residues, mg/kg	Report
Italy, 1995	FS	0.6	grain straw	219 219	≤0.05 ≤0.1	RD/CRLD/ AN/9615958 95609BO2
Italy, 1995	FS	0.6	grain straw	219 219	≤0.05 ≤0.1	RD/CRLD/ AN/9615959 95611BO1
Italy, 1995	FS	0.6	grain straw	219 219	≤0.05 ≤0.1	RD/CRLD/ AN/9615959 95611BO2
Italy, 1995	FS	0.6	grain straw	219 219	≤0.05 ≤0.1	RD/CRLD/ AN/9615992 95611BO1
Italy, 1995	FS	0.6	grain straw	219 219	≤0.05 ≤0.1	RD/CRLD/ AN/9615992 95611BO2
Rye, spring						
Germany, 1985	LS	0.6	foliage grain straw	46 135 135	<0.05 <0.05 <0.05	86-AC-0322 Rs 8501 B2
Rye, winter						
Germany, 1986	LS	0.6	foliage grain straw	214 295 295	<0.05 <0.05 <0.05	87-AC-0172 Rs 8532 B1
Germany, 1986	LS	0.6	foliage grain straw	219 304 304	<0.05 <0.05 <0.05	88-AC-0215 CGD 04-86R
Germany, 1987	LS	0.6	foliage grain straw	230 334 334	<0.05 <0.05 <0.05	88-AC-0215 CGD 10-87R

[1]No report available, only summary table

Sugar cane (Table 16). The data are from a summary table (Anon., 1976) because the full study report was not available to the manufacturer. The use pattern (treatment of cane segments before planting) is such that no residues would be expected at harvest.

Table 16. Residues of guazatine in sugar cane products from treatments before planting. USA, Hawaii, 1976. (Only summary available).

Form	Application No	kg ai/hl	Sample	Residues, mg/kg	Report
LS	1	0.025 (cold solution)	cane bagasse molasses raw sugar	<0.1 <0.1 <0.1 <0.1	1098-55
LS	1	0.01 (hot solution)	cane bagasse molasses raw sugar	<0.1 <0.1 <0.1 <0.1	1098-55

Citrus fruits (Table 17). Trials on oranges (10), mandarins (1), lemons (3) and grapefruit (2) were carried out in Australia (Thornberg, 1980a), Israel (Bodin, 1978), Italy (Thornberg, 1980b) and the USA (Karlsson and Risholm-Sundman, 1988). In some cases the pulp and peel were weighed and analysed separately and the residue in the whole fruit calculated. The results are shown in Table 17.

In two of the trials Bodin (1978) used ^{14}C- and ^{3}H-labelled guazatine acetate to determine the penetration of the active ingredient into treated oranges after storage up to 50 days at +4°C. In two of four trials ethylene was used for degreening; this treatment had no significant influence on the residual content of guazatine.

Table 17. Residues of guazatine in citrus fruits, post-harvest treatment. All single applications of SL formulation.

Fruit, Country, year	Application		Sample	Residues, mg/kg	Storage, days	Report
	kg, ai/hl	Method				
Oranges						
Australia, 1979	0.05	flooding	whole fruit peel pulp	0.3 3 <0.05	7 7 7	80AC0184
Israel, 1978[1]	0.2	dipping	Whole fruit peel white pulp	5.5[2] 17 1.3 0.1	50 50 50	78-08-01
Israel, 1978[1]	0.1	Dipping	Whole fruit peel white pulp	1.8[2] 6.3 0.4 0.03	50 50 50	78-08-01
Israel, 1978[1]	0.2	dipping, degreening with ethylene[3]	Whole fruit peel white pulp	5.5[2] 15 0.7 0.1	50 50 50	78-08-01
Israel, 1978[1]	0.1	dipping, degreening with ethylene[3]	Whole fruit peel white pulp	1.8[2] 4.8 0.1 0.03	50 50 50	78-08-01
Israel, 1988	0.2 (in wax)	dipping	Whole fruit peel pulp	1.8[2] 7[4] <0.05[4]		88AC0343 48526-4-88
Italy, 1978	0.1 (in water)	dipping	Whole fruit peel pulp	0.6 (2), 0.5 4, 0.4, 2 <0.05	2 5 2 5 2	80AC0187 1065-78

Fruit, Country, year	Application		Sample	Residues, mg/kg	Storage, days	Report
	kg, ai/hl	Method				
Italy, 1978	0.2 (in wax)	dipping	whole fruit	0.5 0.6 0.5 0.3 0.2	0 2 5 10 20	80AC0187 1065-78
USA, 1983	0.1 (in water)	spraying	whole fruit peel pulp	0.2[2] 0.7 <0.05		84AC0232
USA, 1983	0.2 (in wax)		whole fruit peel pulp	0.7[2] 3 <0.05		84AC0232[5]
Mandarins						
Australia, 1979	0.05	flooding	whole fruit peel pulp	0.5 2 <0.05	7 7 7	80AC0184
Lemons						
Australia, 1979	0.05	flooding	whole fruit peel pulp	<0.2 2 <0.05	7 7 7	80AC0184
USA	0.1 (in water)	spraying	whole fruit peel pulp	0.45[2] 0.8 0.05		84AC0232[5]
USA	0.2 (in wax)	spraying	whole fruit peel pulp	0.8[2] 1.3 0.13		84AC0232[5]
Grapefruit						
USA, 1983	0.1 (in water)	spraying	whole fruit peel pulp	0.08[2] 0.13 <0.05		84AC0232[5]

Fruit, Country, year	Application		Sample	Residues, mg/kg	Storage, days	Report
	kg, ai/hl	Method				
USA, 1983	0.2 (in wax)	spraying	Whole fruit Peel Pulp	0.33[2] 0.83 0.09		84AC0232[5]

[1]Treatment with ^{3}H- and ^{14}C-labelled guazatine, residues calculated as guazatine equivalents
[2]Residues in whole fruit calculated from residues in pulp and peel
[3]Fruits were drenched with guazatine solution, dried and degreened with gaseous ethylene (10-20 ppm for 24 h).
[4]Mean of 10 fruits
[5]Only summary report available

FATE OF RESIDUES IN STORAGE AND PROCESSING

In storage

In a study of the stability of guazatine residues during storage Bodin (1978) drenched oranges with 0.1 or 0.2 kg ai/hl (1000 or 2000 ppm) of ^{14}C- and ^{3}H-labelled guazatine acetate in water at 12-18°C for 30-60 seconds. Half of the oranges were degreened with ethylene. After drying and waxing, the fruits were stored at 4°C in plastic bags in a refrigerator. Samples were taken after 0, 1, 8, 24 and 50 days. The oranges were separated into yellow peel, white, and pulp, which were pulverized and frozen at -20°C. The results are shown in Table 18. Residue concentrations were reported as mg/kg dry weight. Fruits stored for 50 days at 4°C were reported as being "storage-damaged".

Table 18. Storage stability of guazatine in oranges (Bodin, 1978).

Storage, days	^{14}C or ^{3}H, mg/kg dry wt. as guazatine acetate							
	0.1 kg ai/hl				0.2 kg ai/hl			
	^{14}C deg	^{14}C not deg	^{3}H deg	^{3}H not deg	^{14}C deg	^{14}C not deg	^{3}H deg	^{3}H not deg
Peel								
0	22	13	19	16	53	53	55	51
1	19	41	17	39	68	84	76	93
8	31	36	27	27	60	95	61	96
24	21	28	18	29	83	63	100	69
50	22	25	18	24	54	56	56	74
White								
0	0.41	2.4	0.59	4.4	1.8	1.2	1.7	2.4
1	0.70	0.99	0.69	1.1	1.4	3.5	1.7	4.0
8	0.47	0.96	0.46	1.3	2.6	2.7	3.9	2.9
24	1.2	1.6	0.88	1.4	4.9	5.1	9.9	4.5
50	0.89	3.5	0.77	4.0	4.0	17	4.4	23
Pulp								
0	0.52	0.36	0.25	0.19	0.68	1.2	0.39	1.1
1	0.51	0.02	0.31	0.30	0.94	0.39	5.1	0.27
8	0.21	0.36	0.07	0.27	0.55	0.32	0.39	0.35
24	0.31	0.26	0.15	0.11	0.90	0.78	0.71	0.67
50	0.67	0.77	0.48	0.94	4.3	4.1	5.5	4.5

The stability of guazatine in Zivdar wax treated at 0.1 and 0.2 kg ai/hl and stored in closed vessels at 35°C for three months was studied by Karlsson and Risholm-Sundman (1988). The guazatine concentrations in the wax were 1025 and 1792 mg/kg before storage and 1126 and 2056 mg/kg after three months, the increase in concentration being due to water evaporation. No decomposition of guazatine could be detected.

In processing

A processing study on grapefruit and oranges was reported by Stensiö and Thorstensson (1987). The results are shown in Table 19. The "peel" of the whole fruit is defined as that obtained by hand peeling. "Wet peel" is from machine-peeled fruit where a water spray is constantly used in the peeler. "Dried peel" is produced by the addition of lime, $Ca(OH)_2$, to the wet peel fraction followed by drying in a tunnel dryer.

Table 19. Residues of guazatine acetate in processed fractions of citrus fruit (Stensiö and Thorstensson, 1987), USA.

Fruit	Application, kg ai/hl	Sample	Residue, mg/kg	Processing factor
Grapefruit	0.1	whole fruit	0.08[1]	
		peel	0.13	1.6
		pulp	<0.05	<0.6
		peel oil emulsion	0.18	2.3
		press liquor	<0.06	<0.75
		cold press grapefruit oil	<0.05	<0.6
		juice	<0.05	<0.06
		dried peel	1.2	15
		finisher pulp	<0.05	<0.6
		molasses	1.4	18
Grapefruit	0.2	whole fruit	0.33[1]	
		peel	0.83	2.5
		pulp	0.09	0.3
		peel oil emulsion	0.59	1.8
		press liquor	0.39	1.2
		cold press orange oil	<0.05	<0.15
		juice	<0.05	<0.15
		dried peel	2.1	6.4
		finisher pulp	<0.05	<0.15
		molasses	1.2	3.6
Oranges	0.1	whole fruit	0.2[1]	
		peel	0.7	3.5
		pulp	<0.05	<0.25
		peel oil emulsion	0.1	0.5
		press liquor	0.25	1.3
		cold press orange oil	<0.05	<0.25
		juice	<0.05	<0.25
		wet peel	0.4	2
		dried peel	max. 2.6	13
		finisher pulp	<0.05	<0.25

Fruit	Application, kg ai/hl	Sample	Residue, mg/kg	Processing factor
		molasses	0.33	1.7
Oranges	0.2	whole fruit	0.7[1]	
		peel	3	4.3
		pulp	<0.05	<0.07
		peel oil emulsion	0.49	0.7
		press liquor	0.69	1
		cold press orange oil	<0.05	<0.07
		juice	0.09	0.13
		wet peel	0.83	1.2
		dried peel	max. 3.4	4.9
		finisher pulp	0.1	0.14
		molasses	0.7	1

[1]Residues in whole fruit calculated from residues in pulp and peel

Processing factors for the peel and pulp fractions of the fruit can be calculated from the results of the supervised trials on citrus fruits in Table 17 as shown in Table 20.

Table 20. Residues of guazatine in peel and pulp fractions of citrus fruits.

Fruit	Country	Application, kg ai/hl	Sample	Residue, mg/kg	Ratio to whole fruit
Oranges	Israel	0.2	whole fruit	5.5	
			peel	17	3.1
			white	1.3	0.24
			pulp	0.1	0.02
Oranges	Israel	0.1	whole fruit	1.8	
			peel	6.3	3.5
			white	0.4	0.2
			pulp	0.03	0.02
Oranges	Israel	0.2	whole fruit	5.5	
			peel	15	2.7
			white	0.7	0.13
			pulp	0.1	0.02
Oranges	Israel	0.1	whole fruit	1.8	
			peel	4.8	2.7
			white	0.1	0.06
			pulp	0.03	0.02
Oranges	Isreal	0.2	whole fruit	1.8	
			peel	7	3.9

Fruit	Country	Application, kg ai/hl	Sample	Residue, mg/kg	Ratio to whole fruit
			pulp	<0.05	<0.03
Oranges	Italy	0.1	whole fruit	0.6	
			peel	4	6.7
			pulp	<0.05	<0.08
Oranges	Italy	0.1	whole fruit	0.5	
			peel	2	4
			pulp	<0.05	<0.1
Oranges	Australia	0.05	whole fruit	0.3	
			peel	3	10
			pulp	<0.05	<0.17
Mandarins	Australia	0.05	whole fruit	0.5	
			peel	2	4
			pulp	<0.05	<0.1
Lemons	Australia	0.05	whole fruit	<0.2	
			peel	2	
			pulp	<0.05	
Lemons	USA	0.1	whole fruit	0.45	
			peel	0.8	1.8
			pulp	0.05	0.1
Lemons	USA	0.2	whole fruit	0.8	
			peel	1.3	1.6
			pulp	0.13	0.16

Residues in the edible portion of food commodities

The data on citrus fruits (Tables 17, 19 and 20) show that almost all the residues were found in the peel with a maximum level of 17 mg/kg. The pulp and juice never contained more than 0.13 mg/kg guazatine.

RESIDUES IN FOOD IN COMMERCE OR AT CONSUMPTION

No data were received.

NATIONAL MAXIMUM RESIDUE LIMITS

The following national MRLs were reported. The residues are defined as guazatine.

Country	Commodity	MRL, mg/kg

Country	Commodity	MRL, mg/kg
Australia	Citrus fruits	5
	Melons, except watermelon	5
	Tomato	5
Austria	Citrus fruits	5
Belgium	Fruits	0.1
	Vegetables	0.1
	Potato	0.1
Finland	Citrus fruits	5
Germany	Citrus fruits	5
	Citrus juice	0.5
	Melons	5
	Oil seeds	0.5
	Cereals	0.2
	Other commodities of plant origin	0.05
Italy	Cereal grains	0.1
The Netherlands	Fruits, except citrus	0.1
	Citrus fruits	5
	Melons	5
	Cereal grains	0.1
New Zealand	Citrus fruits	5
Norway	Citrus fruits	5
Spain	Fruits, except citrus	0.1
	Citrus fruits	5
	Melons	5
	Potato	5
Sweden	Citrus fruits	5
	Melons	5
Switzerland	Cereal grains	0.05

APPRAISAL

Guazatine was evaluated by the JMPR in 1978 and 1980, and is now re-evaluated in the CCPR periodic review programme. It is a non-systemic contact fungicide which disturbs the membrane function of fungi. It controls a wide range of seed-borne diseases of cereals, e.g. seedling blight *(fusarium spp.)*, glume blotch *(septoria)*, common bunt *(tilletia spp.)*, common root rot *(helminthosporium)* and smut *(ustilago)*. On citrus fruit, guazatine is used as a bulk dip after harvest, in the packing line as a spray and in washing installations to disinfect the process water. It controls sour rot *(geotrichum candidum)*, green mould *(penicillium digitatum)* and blue mould *(penicillium italicum).*

Guazatine is a mixture of reaction products from polyamines, comprising mainly octamethylenediamine, iminodi(octamethylene)diamine, octamethylenebis(imino-octamethylene)diamine, and carbamonitrile. A coding system is used for the compounds that make up guazatine in which "N" represents any amino group. Thus NN stands for H_2N-$(CH_2)_8$-NH_2 , NNN stands for H_2N-$(CH_2)_8$-NH-$(CH_2)_8$-NH_2 and so on. "G" stands for any amino group (NH or NH_2) of the above which is guanidated. For example GG stands for H_2N-C(NH)NH-$(CH_2)_8$-NH-C(NH)-NH_2.

The fate of residues has been studied in animals, plants and soil.

Studies on rats and lactating cows showed poor absorption from the gastrointestinal tract, rapid elimination mainly in the faeces (>90%), excretion largely as the unchanged parent mixture and no accumulation in any organs, tissues or milk.

When cows were dosed daily with 0.5 mg/kg bw for 10.5 days, 93% of the administered radioactivity was recovered in the faeces as unchanged guazatine, and the low levels in plasma indicated minimal absorption. ^{14}C in the milk and plasma, expressed as guazatine, reached plateau levels of about 0.02 and 0.015 mg/l respectively by day 3 in milk and day 6 in plasma. Following slaughter after the last dose residues of about 0.08 mg/kg were found in the liver and kidney with only very low levels in other edible tissues (<0.02 mg/kg in skeletal muscle and fat).

Adequate metabolism studies with full characterization of the metabolites in farm animals, an animal transfer study on ruminants and an analytical method for commodities of animal origin were not submitted. The Meeting was therefore unable to establish a definition of the residue of guazatine in animal products and could not estimate maximum residue levels for products of animal origin.

When wheat seeds were dressed with [^{14}C]guazatine at 1.05 g ai/kg seed there was no difference between the total radioactive residue (TRR) levels in the harvested grain, straw or chaff from the treated and the control plots. The method of application was according to GAP.

The foliar application of [^{14}C]guazatine to wheat at 1.1 kg ai/ha, 11 weeks before harvest, resulted in mean TRRs of 29 mg/kg guazatine equivalents in the straw, 18 mg/kg in the chaff, and 0.8 mg/kg in the grain.

When [^{14}C]guazatine was applied to the leaf surface or the fruit of apples (brushing with 0.05 or 0.1 kg ai/hl) its translocation was extremely limited. Autoradiography showed no observable movement in the leaves or fruit and this was confirmed by quantitative determination of the TRR: 87% of the applied radioactivity was recovered from the leaves after 12 weeks, 66% from the surface and 21% from the leaf tissues (61% was identified as the parent mixture). In the fruit 62% of the TRR was located on the surface and 38% in the tissues after 12 weeks, with 81% of the TRR identified as the parent. The remainder comprised a major photodegradation product (4.5%), other extractable compounds (9.7%), and unextractable residues (5.2%).

The uptake of guazatine residues from soil by soya beans and rice plants was investigated by treating soils with 5 mg/kg of [^{14}C]guazatine and planting soya bean and rice plants after 26 weeks.

Four weeks after planting, the TRR in soya beans amounted to only 0.08% of the applied radioactivity in the aerial part and 0.12% in the whole plant. The residues expressed as guazatine equivalents on a dry weight basis were 2.8 mg/kg in the aerial part and 3.7 mg/kg in the whole plant. The pods contained 0.052 mg/kg on a dry weight basis 9 weeks after planting.

Guazatine residues taken up from flooded soil were low in the whole rice plant, which absorbed only 0.13% of the applied ^{14}C (0.57 mg/kg on a dry weight basis) during a period of four weeks, with 0.05 % of the applied radioactivity or 0.23 mg/kg (dry weight) in the shoot.

Guazatine has been shown to be metabolized in about 100 days when applied to wheat seeds planted in soil, via deguanidation and subsequent mineralization. The test system had a substantial influence on the degradation time.

When guazatine was applied to wheat seeds which were subsequently planted in soil and the soil leached to simulate rainfall, the guazatine components were found to be associated with the seeds or the soil surrounding the seeds. The compounds that had moved from the seeds to the soil showed no tendency to migrate. Significant mineralization to carbon dioxide occurred during the leaching period.

The Meeting concluded that these studies were adequate for the use of guazatine for the seed treatment of cereals, and that no further studies on rotational crops were necessary for such uses.

The use of such a complex mixture as guazatine presents a problem in choosing a residue analytical method. It is not considered practical to attempt the determination of all the components so some alternative is necessary. Two approaches may be applicable.

1. Development of a "total residue" method by conversion to a single compound.

2. The choice of a major component as a "marker", with the inclusion of a correction factor to give the total residue.

Many of the residue studies used the first approach, involving the hydrolysis of residues to bis(8-amino-octyl)amine (NNN) and its determination either directly or after derivatization. This method was used, e.g., for the analysis of citrus fruits, where the LODs (expressed as guazatine) were 0.05 mg/kg for finisher pulp, 0.2 mg/kg for wet peel and 1 mg/kg for dried peel. The metabolites are determined by the total residue method together with the parent material.

Better results were achieved with cereals, however, by using the marker GG (octane-1,8-diyldiguanidine, $H_2N\text{-}C(NH)NH\text{-}(CH_2)_8\text{-}NH\text{-}C(NH)\text{-}NH_2$), one of the major guazatine components, for quantification. This method incorporates a correction factor to allow for the fact that GG represents only 30% of the total guazatine. The homologue GG-C6 (1,6-diguanidinohexane, $H_2N\text{-}C(NH)NH\text{-}(CH_2)_6\text{-}NH\text{-}C(NH)\text{-}NH_2$), is used as an internal standard. The analytical method for grain and straw consists in extraction of samples fortified with the internal standard with hot 1M HCL, clean-up on a cation exchange column, derivatization with hexafluoroacetylacetone (HFAA), clean-up on basic Al_2O_3, and determination of the HFAA derivatives of GG and the internal standard GG-C_6 by GC-MS.

Samples fortified with guazatine showed LODs of 0.05 mg/kg for cereal grains and 0.1 mg/kg for straw with recoveries of 88% and 94% respectively. The lowest fortification levels at the LOD of the marker GG were also 0.05 mg/kg for grain and 0.1 mg/kg for straw (recoveries: grain 97%, straw 82%).

The Meeting concluded that 0.05 mg/kg is a practical limit of determination for GG.

The justification for the choice of GG as representative of the total guazatine residues in cereals has been supported by the following facts.

1. Guazatine shows low uptake and translocation in cereals. This is consistent with the lack of detectable residues reported in crops after seed treatments.

2. Where the material has been applied as a foliar spray on dwarf apples trees there is little evidence of significant metabolism or hence of changes in the proportions of the components of the guazatine mixture.

3. In a situation where metabolism is demonstrably occurring (see below), GG remains a significant component after 29 days.

Evidence for GG still being present under "metabolizing" conditions comes from an aerobic soil degradation study. In this, a mixture of GG, GN and GGG was applied to seed surfaces, and the seeds were planted in soil in metabolism vessels. Most of the seeds germinated. It was possible to distinguish the seeds from the soil and extract the seeds separately up to 29 days after planting. Analysis of these extracts indicated a change in the profile of components present on the seed with GGG levels decreasing. This is consistent with the generation of $^{14}CO_2$ in the study. However at day 29 GG was still the predominant single compound on the seed, despite the degradation which had been occurring at the seed surface or in the soil in contact with it.

On this basis, it is considered that GG represents a satisfactory marker compound to represent guazatine residues in seed-treated cereals.

The storage stability of analytical samples was investigated by storing analysed samples of wheat grain, ears and straw at -20EC and re-analysing them after two years. The study was not satisfactory as an unvalidated analytical method was used.

Definition of the residue. The metabolism of guazatine in animals has not been fully elucidated, and the Meeting concluded that the residue of guazatine in products of animal origin could not be satisfactorily defined.

The metabolism of guazatine in plants has also not been fully characterized. The main uses of guazatine are for the seed treatment of cereals and the post-harvest protection of citrus fruits. The Meeting concluded that the available studies were adequate only for the seed treatment of cereals. Should further uses (e.g. foliar spray or treatment of plants other than cereals) be planned in future, detailed metabolism studies would be required.

Guazatine has been determined by a total residue method based on conversion to the corresponding triamine, bis(8-amino-octyl)amine, which also occurs as a metabolite. Modern analytical methods using octane-1,8-diyldiguanidine (GG), one of the main components of guazatine, as a marker are more specific.

The Meeting concluded that the residue should be defined for enforcement purposes as "octane-1,8-diyldiguanidine" (GG). Assuming that the content of GG is 30% of the total guazatine content, the GG content should be multiplied by 3 for risk assessment purposes for commodities of plant origin.

Definition of the residue for enforcement purposes: octane-1,8-diyldiguanidine (GG), expressed as octane-1,8-diyldiguanidine.

Definition of the residue for risk assessment purposes: guazatine.

Supervised trials

Citrus fruits. Concentrations of 0.05 to 0.2 kg ai/hl water or 0.3 kg ai/hl wax are registered for post-harvest treatment.

In Australia, guazatine is registered for the post-harvest treatment of citrus fruits with 0.052 kg ai/hl. Three residue trials according to GAP (one each on oranges, mandarins and lemons) were reported and showed residues of <0.2, 0.3 and 0.5 mg/kg (calculated as guazatine) in the whole fruit.

South African GAP specifies 0.3 kg ai/hl in wax for the treatment of citrus fruits. Five trials (3 on oranges, one each on lemons and grapefruit) at the lower rate of 0.2 kg ai/hl in wax were reported. The residues in the whole fruit ranged from 0.33 to 1.8 mg/kg, calculated as guazatine. These results and the data on the validation of the method were submitted only as summaries.

After dipping oranges in water with 0.2 kg ai/hl guazatine, residues of 5.5 mg/kg were calculated in the whole fruit (2 trials). These results are inconsistent with the results found after waxing and indicate a more critical residue situation. Furthermore, no data were available on residues in small citrus fruits (e.g. mandarins) after treatment with 0.2 kg ai/hl.

The Meeting concluded that the residue data were not adequate for citrus fruits as a major crop and recommended the withdrawal of the existing CXL of 5 mg/kg.

Tomatoes and melons, except watermelons. Post-harvest uses of guazatine exist in Australia but no residue data were received.

No maximum residue level could be estimated for tomatoes, and the Meeting recommended the withdrawal of the existing CXL of 5 mg/kg for melons, except watermelon.

Pineapples and potatoes. Since no residue data or information on GAP were received, the Meeting recommended the withdrawal of the existing CXLs of 0.1* mg/kg for pineapple and potato.

Cereal grains. The use of guazatine for seed treatment is registered in many countries with application rates from 0.05 to 1.05 g ai/kg seed (mainly 0.45-0.6 g ai/kg). A total of 84 supervised trials with treatments at 0.4, 0.6, 0.8, 0.9, 1, 1.2 or 1.5 g ai/kg seed were reported to the Meeting. The samples from 61 trials carried out from 1972 to 1987 were analysed by an unvalidated analytical method and could not be used for evaluation. Valid results from 23 trials carried out in 1994/95 on wheat in France (7), Germany (6) and Italy (10) were submitted. No residues were found above the LOD of 0.05 mg/kg, calculated as guazatine.

In view of the non-systemic character and particular use pattern of guazatine as a seed treatment, the Meeting concluded that the residue in cereal grains was "essentially zero" and estimated an STMR of 0 mg/kg.

The Meeting estimated a maximum residue level of 0.05* mg/kg expressed as GG for cereal grains as a practical limit of determination.

Sugar cane. Guazatine is registered in South Africa for the treatment of plant segments before planting with a solution of 0.08 kg ai/hl water. Only two trials, not complying with GAP, were reported. Sugar cane was treated in Hawaii with solutions of 0.01 or 0.025 kg ai/hl. Residues in cane, bagasse, molasses and raw sugar were reported as <0.1 mg/kg. The report was submitted only as a summary with little information (e.g. the PHI and analytical method were not stated).

The Meeting recommended the withdrawal of the existing CXL (0.1 mg/kg).

Rape seed. The use of guazatine as a foliar spray is registered in Germany but no residue data were received. No maximum residue level could be estimated.

Straw and fodder of cereal grains. After treatment of wheat with 0.6-0.8 kg ai/kg seed the residues found in 21 trials carried out in 1994/95 in France (7), Germany (6) and Italy (8) were all <0.1 mg/kg calculated as guazatine.

As there was no residue definition for guazatine in animal products, the Meeting did not recommend an MRL for the straw and fodder of cereal grains as a feed item.

Animal products. No transfer study was carried out on ruminants, no definition of the residue in products of animal origin could be proposed, and no maximum residue levels were estimated for any animal feed items.

The Meeting concluded that there was insufficient information to estimate maximum residue levels for guazatine in products of animal origin.

No feeding or metabolism studies were reported for laying hens. As no residues occur in cereal grains after seed treatment, the Meeting concluded that further studies and the estimation of maximum residue levels for residues in poultry commodities resulting from seed treatment were not necessary.

A study of the storage stability of radiolabelled guazatine on oranges after drenching with 0.1 or 0.2 kg ai/hl showed no decrease of the residues after 50 days.

The results of commercial processing studies on citrus fruits indicate that the residues are on the peel surface. Processing factors calculated for dried peel were 4.9, 6.4, 13 and 15, mean 9.8, median 9.7, and for molasses 1, 1.7, 3.6 and 18, mean 6.1, median 2.7. There was a clear reduction of the residue during processing to pulp and juice. The analysis of fresh peel in 15 supervised trials showed ratios of the residues in the peel to those in the whole fruit ("processing factors") of 1.6 (2), 1.8, 2.5, 2.7 (2), 3.1, 3.5 (2), 3.9, 4 (2), 4.3, 6.7 and 10. with a mean of 3.7 and a median of 3.5.

Residues in the edible portions of citrus fruits were low. After treatment according to GAP, most pulp and juice samples contained guazatine residues at or about the LOD (#0.05 mg/kg) and never more than 0.13 mg/kg.

No information was provided on residues in commodities in commerce or at consumption.

The Meeting estimated the maximum residue level shown in Annex I (Part 2). As the Meeting withdrew the ADI for guazatine this is recorded only as a Guideline Level.

FURTHER WORK OR INFORMATION

Desirable

Any further evaluations for uses apart from the seed treatment of cereals would require the following data.

1. Clarification of the metabolism of all major components in ruminants.

2. Animal transfer studies on ruminants including an analytical method for the determination of residues in products of animal origin.

3. Clarification of the metabolism of all major components in plants.

RECOMMENDATIONS

The Meeting estimated the maximum residue level shown below. As the Meeting withdrew the ADI for guazatine this is recorded only as a Guideline Level. Other previous estimates were withdrawn.

Pesticide (Codex ref. no.)	Commodity	GL, mg/kg		Previous MRL, mg/kg	STMR, mg/kg
	CCN	Name			
Guazatine	FC 0001	Citrus fruits	W	5 Po	
(114)	FI 0353	Pineapple	W	0.1*	
	GC 0080	Cereal grains	0.05*	0.1*	0
	GS 0659	Sugar cane	W	0.1*	
	VC 0046	Melons (except Watermelon)	W	5 Po	

	VR 0589	Potato	W	0.1*	
	Residue for GLs: octane-1,8-diyldiguanidine ("GG"), expressed as octane-1,8-diyldiguanidine for STMRs: guazatine				

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