CYROMAZINE (169)

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EXPLANATION

Cyromazine, a selective insecticide, was first evaluated by JMPR in 1990, subsequently in 1991 and 1992 for residues, and in 2006 for toxicity when an ADI of 0–0.06 mg/kg bw and an ARfD of 0.1 mg/kg bw were established. It was again evaluated by JMPR in 2007 under the periodic review programme. The 2007 JMPR recommended a residue definition of cyromazine for plant and animal commodities both for compliance with the MRL and for estimation of dietary intake; and, recommended 24 maximum residue levels and withdrew three previous recommendations.

The current Meeting received information on methods of analysis, storage stability and supervised trials to support additional maximum residue levels for cyromazine.

METHODS OF RESIDUE ANALYSIS

Analytical methods

The 2007 JMPR reviewed four analytical methods for determination of cyromazine in vegetables and concluded they are satisfactorily validated.

The current Meeting received information on analytical methods used in the supervised residue trial studies.

Snap beans

Snap bean samples were analysed for cyromazine using the working method involving extraction of cyromazine by refluxing chopped snap bean samples in 10% water:methanol for 2 hours (Starner, 2007). An aliquot of the extract was evaporated to the aqueous, diluted with 0.1M HCl and cleaned up by partition with dichloromethane followed by partition with hexane. The sample was further cleaned up by cation-exchange chromatography followed by anion-exchange chromatography. Cyromazine residues were determined by gas chromatography with NPD detection. This method was validated at the fortification levels of 0.1–2.0 mg/kg for analysis of snap beans. The lowest level of method validation was 0.10 mg/kg for cyromazine.

Table 1 Recoveries by GC-NPD method for the determination of cyromazine in snap bean (pods and seeds (B3909.02)

Matrix	Fortification level (mg/kg)	Recovery rate (%)		RSD (%)	n
		Individual results	Mean		
Snap bean (pods and seeds)	0.10	96, 88, 82	89	8	3
	1.0	84, 102, 90	92	10	3
	2.0	95, 101, 94, 100, 115, 95	100	8	6
	0.10–2.0	82–115	95	9	12

French bean

French bean samples obtained from a trial in Senegal were analysed using a published HPLC/MS method with some modifications (Mende, 2005).

The HPLC/MS method involves extraction of cyromazine with a mixture of methanol and water (1:1, v/v) and, after centrifugation and clean-up by Envi-Carb column chromatography (elution using methanol containing 5% (v/v) aqueous ammonia), determination of cyromazine using HPLC/MS (parent ion). The method was validated for cyromazine in French beans at two fortification levels. The lowest level of fortification was 0.02 mg/kg.

Table 2 Recoveries by HPLC/MS method for the determination of cyromazine in French beans

Matrix	Fortification level (mg/kg)	Recovery rate (%)		RSD (%)	n
		Individual results	Mean		
French bean	0.02	117, 100, 97, 102, 98	103	8	5
	0.50	95, 98, 91, 98, 96	96	3	5
	0.02–0.50	91–117	99	7	10

French bean samples obtained from trials in Kenya were analysed using an HPLC/MS/MS method (method P-14.104.01) (Rzepka, 2005).

Cyromazine residues were extracted from 15 g of sample with acetonitrile, and the acetonitrile phase was cleaned up with a dispersive solid-phase extraction (dispersive SPE). The acetonitrile extract was diluted in water and cyromazine was determined by means of liquid chromatography with tandem mass spectrometric detection. The method was validated for French beans at two fortification levels. The lowest level of fortification was 0.01 mg/kg.

Table3. Recoveries by HPLC/MS/MS method for the determination of cyromazine in French beans

Matrix	Fortification level (mg/kg)	Recovery rate (%)		n
		Individual results	Mean	
French bean	0.01	78		1
	0.10	84		1
	0.01–0.10	78–84	81	2

Lima beans

Lima bean samples were analysed using the method "Determination of Cyromazine in Bean (Lima)" (2001) based on reference method "Analytical Method for the Determination of Cyromazine and its Metabolite Melamine Residues in Crops by Gas Chromatography with a Nitrogen/Phosphorous Detector in the Nitrogen Specific Mode", Method No. AG-621, reviewed by the 2004 JMPR, with minor modification (Starner, 2004). This method was validated at the fortification level of 0.05–5.0 mg/kg in lima beans (seeds). The lowest level of fortification was 0.05 mg/kg.

Table 4 Recoveries by GC-NPD method for the determination of cyromazine in lima beans (seeds)

Matrix	Fortification level (mg/kg)	Recovery rate (%)		RSD (%)	n
		Individual results	Mean		
Lima bean (seeds)	0.050	74, 70, 87, 69, 68, 97, 83, 66, 86, 87, 95	80	14	11
	0.5	90, 69, 72	77	15	3
	5.0	104, 115, 106	108	5	3
	0.05–5.0	69–115	85	18	17

Stability of residues in stored analytical samples

The 2007 JMPR concluded that cyromazine at the fortification level of 1 mg/kg was stable in haricot beans for at least two years when stored at ≤ -18 °C. Samples from the US supervised trials were stored frozen no longer than one year and French bean samples from the supervised trials in Senegal and Kenya were stored no longer than four months.

USE PATTERN

The Meeting received approved labels in the United States of America. Information on registered formulations, application methods and dosage rates of cyromazine for uses on the crops for which supervised trial data were provided is summarized in Table 5.

Table 5 Registered uses of cyromazine related to supervised trials

Crop	Country	Formulation		Application					PHI days
		g ai/L or g ai/kg	type	Method	g ai/ha	L/ha	Interval days	Max no. (max g ai/ha) in season	
Beans, dried varieties except cow peas and soybeans	USA	750	WP	Foliar	140	Aerial Min. 46.8 Ground Min. 93.6	7	6 (841)	7
Beans, succulent (only *Phaseolus* spp)	USA	750	WP	Foliar	140	Aerial Min. 46.8 Ground Min. 93.6	7	6 (841)	7
French bean	Kenya	750	WP	Foliar	225	200–800	10–14	3	14

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

The Meeting received information on supervised field trials of cyromazine on the following crops:

Crop group	Commodity	Country	Table No.
Legume vegetables	Snap beans	USA	Table 6
	French beans	Senegal, Kenya	Table 7
	Lima beans	USA	Table 8

Legume vegetables

Common bean (Snap bean and French bean)

Eight trials were conducted in the USA during the 2002 growing season, three in Georgia, one in New York, and two each in Wisconsin and Washington (Starner, 2007). In each trial, six foliar applications of WP formulation of cyromazine at approximately 140 g ai/ha each were applied to snap beans for a total of approximately 841 g ai/ha. No adjuvant was added to the spray mixtures. The applications were made 6 to 8 days apart, and samples were collected 6 to 7 days following the final application.

During the trial in New York State drought conditions occurred during July and August, and irrigation was used.

Samples were analysed for residues of cyromazine using the method, "Determination of Cyromazine in Bean (snap)" derived from Ciba-Geigy Analytical Method No. AG-621, "Analytical Method for the Determination of Cyromazine and its Metabolite Melamine Residues in Crops by Gas Chromatography with a Nitrogen/Phosphorus Detector in the Nitrogen Specific Mode".

The longest storage duration of field-treated samples was 140 days.

Table 6 Cyromazine residues in snap bean (pods and immature seeds) from supervised trials in the USA

Snap bean Location, year (variety)	Form	Application				PHI, days	Cyromazine mg/kg (mean of replicates)	Reference Trial no
		g ai/ha	Water, L/ha	No	Total/ season, g ai/ha			
US GAP (max)	WP	140	Aerial Min. 46.8 Ground Min 93.6	6	841	7		

Snap bean Location, year (variety)	Form	Application g ai/ha	Water, L/ha	No	Total/ season, g ai/ha	PHI, days	Cyromazine mg/kg (mean of replicates)	Reference Trial no
Tifton, GA 2002 (RWM 536) Last app. 5/31 Harvest 6/6	WP	140 140 141 142 142 141	187 186 188 475 475 472	6	847	6	1.26	B3909.02 02-GA*15
Tifton, GA 2002 (EX8104389) Last app. 5/31 Harvest 6/6	WP	140 141 140 141 143 142	186 188 187 472 479 475	6	848	6	1.01	B3909.02 02-GA*16
Tifton, GA 2002 (EX08100518) Last app. 5/31 Harvest 6/6	WP	141 140 141 141 142 142	188 187 188 470 477 477	6	848	6	1.13	B3909.02 02-GA*17
Freeville, NY 2002 (Labrador)	WP	139 139 143 142 146 145	278 277 286 284 291 290	6	854	7	1.31	B3909.02 02-NY22
Arlington, WI 2002 (Hystyle) Last app. 7/23 Harvest 7/30	WP	141 141 141 134 141 140	289 290 297 292 297 294	6	840	7	1.17	B3909.02 02-WI25
Arlington, WI 2002 (Bush Blue Lake 156) Last app. 7/23 Harvest 7/30	WP	141 138 139 141 142 141	286 288 290 294 296 294	6	843	7	0.896	B3909.02 02-WI26
Moxee, WA 2002 (Florence) Last app. 8/1 Harvest 8/7	WP	146 141 148 142 143 136	406 396 415 399 403 382	6	856	6	0.599	B3909.02 02-WA*39
Moxee, WA 2002 (Jade) Last app. 8/8 Harvest 8/14	WP	137 139 139 138 141 136	382 388 391 387 395 380	6	829	6	0.799	B3909.02 02-WA*40

A total of five trials were conducted on French beans: two in Senegal (one site, Saint-Louis) and three in Kenya (three sites: Mwea, Thika and Naivasha) in 2003 and 2004. A combination of several plant protection products including cyromazine as well as metalaxyl-M, abamectin, lambda-cyhalothrin, chlorothalonil and difenoconazole (combination 1) was used in each country. Cyromazine in Combination 1 was applied three times at a rate of 225 g ai/ha. (Delhove, 2005)

Trial performed in Senegal (SE/HV/2004/03)

Two trials were performed in Senegal (one site) but at two different sowing periods: November 2003 and January 2004. Combination 1 was applied between February and March 2004 only on French

beans sowed in January 2004. Treatments were applied with a 'tractor-mounted boom sprayer with a volume of application of 800 L/ha at all crop stages.

For the plot treated with cyromazine, whole French beans of commercial quality were collected from the central rows, mixed, put in bags, and bags were sealed and deep frozen (-18 °C).

The storage duration of field-treated samples was 55 days.

Trials performed in Kenya (KE/FB/2004/01, 02 & 03):

Three trials were performed in Kenya on three sites (Mwea, Thika and Naivasha). There were no replicates within one site in 2004. Applications were performed with a band sprayer mounted tractor (knapsack sprayers). The maximum application volume at full development of the crop was fixed to be at 600 L/ha. This application volume, however, was only used during the last spray application. Actual applications went from 200 L/ha to 500–600 L/ha. Cyromazine, part of combination 1, was applied in all three sites.

At all three sites, French beans of commercial quality (fine beans) sampled for analysis were only taken from the inner part of the plots. Beans were harvested manually, collected in buckets and labelled. The harvested amount of each plot was mixed and a randomised sample of 8 kg per plot was taken. Towards the end of harvesting the sample size was reduced to 4 kg per plot according to the needs of the residue laboratory. The field samples were packed into cool boxes and -frozen (-18 °C). One kg of each sample was homogenized for analysis.

Beans were sown on 26th July (Mwea site) and 10th August 2004 (Thika and Naivasha). First sampling was achieved in between 53 and 61 days after the sowing dates, which corresponds to September–October–November 2004).

The first shipment sent out mid-October contained the samples taken from the Mwea trial site, the second shipment on 1st November 2004, the samples from Thika and Naivasha.

Table 7 Cyromazine residues in French beans from supervised trials in Senegal and Kenya

Snap bean Location, year (variety)	Form	Application g ai/ha	Water, L/ha	No	PHI, days	Cyromazine mg/kg	Reference Trial no
GAP, Kenya	WP	225	200–800	3	14		
St Lous, Senegal	WP	160	800	3	3 7	0.35 0.23	SE/HV/2004/03
Mwea, Kenya	WP	225	600	3	3 7 10 14	1.3 0.99 0.50 0.28	KE/FB/2004/01
Thika, Kenya	WP	225	600	3	3 7 10 14	1.3 0.81 0.60 0.24	**KE/FB/2004/02**
Naivash, Kenya	WP	225	600	3	7 10 14	0.73 0.50 0.56	**KE/FB/2004/03**

Lima beans

Three supervised field trials were conducted in Maryland, New Jersey and California (one each) in 2000 (Starner, 2004). Each treated plot received six foliar applications at a rate of approximately 140 g ai/ha per application, for a total of approximately 841 g ai/ha. Each application was made 6 to 8 days apart, and timed so that mature lima beans could be harvested approximately 7 days after the final application.

In each trial two samples of lima been pods were collected at the proper harvest quality. Beans were shelled with machine, and seeds were analysed while the pods were discarded. The maximum storage interval for field-treated samples in this study was 307 days.

Table 8 Cyromazine residues in lima bean (immature seeds) from supervised trials in the USA

Snap bean Location, year (variety)	Form	Application				PHI, days	Cyromazine mg/kg (mean in parentheses)	Reference Trial no
		g ai/ha	Water, L/ha	No	Total/ season, g ai/ha			
US GAP (max)	WP	140	Aerial Min. 46.8 Ground Min 93.6	6	841	7		
Salisbury, MD 2000	WP	145 142 143 142 143 143	486 481 482 479 481 482	6	860	7	0.10 0.11 (0.105)	A3908.00-MD05
Bridgeton, NJ 2000	WP	141 137 139 133 136 133	186 188 187 472 479 475	6	819	8	< 0.05 < 0.05 (< 0.05)	A3908.00-NJ11
Madera, CA 2000	WP	139 138 139 139 139 140	188 187 188 470 477 477	6	834	7	0.19 0.28 (0.235)	A3908.00-CA103

APPRAISAL

Cyromazine was first evaluated by JMPR in 1990 and subsequently in 1991 and 1992 for residues and in 2006 for toxicity when an ADI of 0–0.06 mg/kg bw and an ARfD of 0.1 mg/kg bw were established. It was again evaluated by JMPR in 2007 under the periodic review programme. The 2007 JMPR recommended cyromazine as the residue definition for plant and animal commodities both for compliance with the MRL and for estimation of dietary intake.

At its Forty-third Session, the CCPR included cyromazine in the Priority List to be evaluated by the current Meeting for additional maximum residue levels.

The current Meeting received information on methods of analysis, storage stability and supervised trials to support additional maximum residue levels for cyromazine. The current Meeting also received a request to consider extending the Codex MRL for beans (dry) to chick pea and lentil.

Methods of analysis

The Meeting received information on the analytical methods used in the supervised residue trials submitted to the current Meeting. These methods used were either GC/NPD, HPLC/MS or HPLC/MS/MS. They were validated successfully for determining cyromazine in snap bean (pods and immature seeds), French bean and lima bean with mean recoveries in the acceptable range of 70–110% with RDSs less than 20%.

Stability of pesticide residues in stored analytical samples

The 2007 JMPR concluded that cyromazine at the fortification level of 1 mg/kg was stable in haricot beans for at least two years when stored at ≤ -18 °C. Samples from the US supervised trials were

stored frozen no longer than one year and French bean samples from the supervised trials in Senegal and Kenya were stored no longer than four months.

Results of supervised residue trials on crops

The Meeting received information on supervised on supervised trials of cyromazine on common bean (pods and/or immature seeds), lima bean (immature seeds) and beans (dry).

The OECD MRL calculator was used as a tool to assist in the estimation of maximum residue levels from the selected residue data set obtained from the supervised residue trials. As a first step, the Meeting reviewed trial conditions and other relevant factors related to each data set to arrive at a best estimate of the maximum residue level using expert judgement. Then, the OECD calculator was employed. If the statistical calculation spreadsheet suggested a different value, a brief explanation of the derivation was supplied.

Legume vegetables

Common bean (pods and/or immature seeds)

The Meeting received information on supervised residue trials conducted in the USA, Senegal and Kenya.

Trials were conducted on common bean (snap bean) in four locations in the USA. However, the dates of last application and harvest were either the same or only slightly different at the same locations, and as the variability between varieties is not considered significant for snap beans, the Meeting considered that there were only four valid trials in accordance with US GAP (6 applications at 140 g ai/ha, PHI 7 days). Residues of cyromazine in snap beans (pods and seeds) from these trials were: 0.80, 1.2, 1.3 and 1.3 mg/kg.

A total of three trials were conducted in Senegal (1) and Kenya (3) on common bean (French bean). Residues from the trials according to GAP in Kenya (maximum of three applications with 10-14 day interval, maximum rate of 225 g ai/ha for 200–800 L/ha, a PHI of 14 days) in ranked order, were: 0.24, 0.28 and 0.56 mg/kg.

As the GAP in the USA and that in Kenya are significantly different from each other, the Meeting concluded that data available were insufficient to estimate a maximum residue level for common beans.

Lima bean

Another three trials were reported for lima beans from the USA. Residues of cyromazine in lima bean (immature beans) from trials according to US GAP were: < 0.05, 0.11 and 0.24 mg/kg.

The 2004 JMPR estimated a maximum residue level of 1 mg/kg, STMR of 0.23 mg/kg and HR of 0.58 mg/kg for lima beans (young pods and/or immature beans) based on the six trials from the USA on lima beans in pods. The current Meeting considered that the previous recommendation for lima beans (young pods and/or immature beans) was sufficiently high to cover lima bean immature beans.

Pulses

The Meeting received information on nine trials from a number of states in the USA conducted in 1998 on various kinds of dry beans. Eight of these trials had been provided to the 2004 JMPR which estimated, on the basis of these eight trials and one additional trial in the USA, a maximum residue level of 3 mg/kg for beans (dry), which were adopted as Codex MRL. An STMR of 1.0 mg/kg was also estimated.

Trials were conducted in the USA in 1998 on black-eyed pea (cow pea), pinto bean, navy bean, kidney bean and great northern bean with comparable residue results and US GAP is for all "*dried varieties of beans except cow peas and soya beans*". The current Meeting therefore concluded

that it was appropriate to extend the previous recommendation for beans (dry) to chick-pea (dry), lentil (dry) and lupin (dry).

RECOMMENDATIONS

On the basis of the data from supervised trials, the Meeting concluded that the residue levels listed below are suitable for establishing maximum residue limits and for IEDI assessment.

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for plant and animal commodities: *Cyromazine.*

Commodity		Recommended MRL, mg/kg		STMR or STMR-P (mg/kg)	HR or HR-P (mg/kg)
CCN	Name	New	Previous		
VD 0524	Chick-pea (dry)	3	-	1.0	-
VD 0533	Lentil (dry)	3	-	1.0	-
VD 0545	Lupin (dry)	3	-	1.0	-

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Daily Intakes (IEDIs) of cyromazine were calculated for the 13 GEMS/Food cluster diets using STMRs estimated by the current Meeting (Annex 3 of the 2012 JMPR Report). The ADI is 0–0.06 mg/kg bw and the calculated IEDIs were 0-4 % of the maximum ADI. The Meeting concluded that the long-term intake of residues of cyromazine resulting from the uses considered by the 2007 and current JMPR is unlikely to present a public health concern.

Short-term intake

The International Estimated Short-Term Intakes (IESTI) of cyromazine were calculated for food commodities and their processed commodities using HRs/HR-Ps or STMRs/STMR-Ps estimated by the current Meeting (see Annex 4 of the 2012 JMPR Report). The ARfD is 0.1 mg/kg and the calculated IESTIs were 3–20 % of the ARfD. The Meeting concluded that the short-term intake of residues of cyromazine, when used in ways that have been considered by the JMPR, is unlikely to present a public health concern.

REFERENCES

Code	Authors	Year	Title, Institute, Report reference
	FAO/WHO	2007	Pesticide residues in food—1007, Evaluation 2007, Part I, FAO Plant Production and Protection Paper 191
No number	Delhove, G	2005	Report on residues trials in Senegal and Kenya on French bean 25 February 2005
20041064/02-RP	Mende, P	2005	Determination of Pesticide Residues in Green Beans from Senegal (Second Period of Sowing) GAB Biotechnologie GmbH & GAB Analytik GmbH, Germany, GLP, Not Published
No number	Rzepka, S	2005	Analytical and Advisory services relating to the determination of residues in fresh fruits and vegetables exported by ACP countries with the view to ensure compliance with the European Regulation, 2nd sequence-Ref PIP N° 0106 (French Beans, season 2004),, issued February 2005 Specht and Partner n° PIP-0402 Az.G04-0102. GLP, Not Published

Code	Authors	Year	Title, Institute, Report reference
IR-4 PR No. A3908	Starner, VR	2004	Cyromazine: Magnitude of the Residue on Bean (Lima)
IR-4 PR No. B3909	Starner, VR	2007	Cyromazine: Magnitude of the Residue on Snap Bean (Edible-Podded)