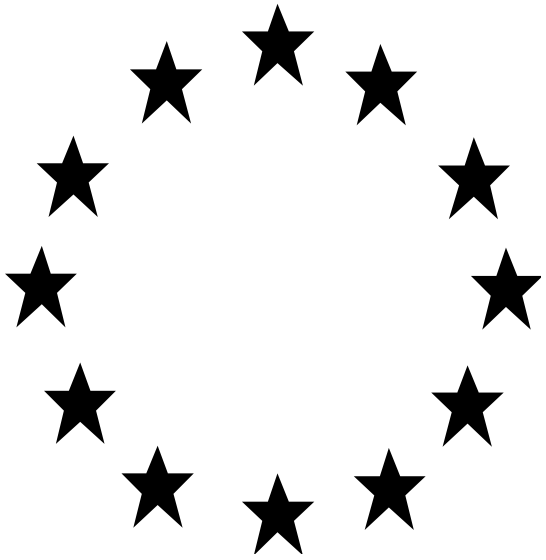


Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Germany for the existing active substance

CHLORIDAZON

of the third stage (part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 3, Annex B, B.7

July 2005

Annex B

Chloridazon

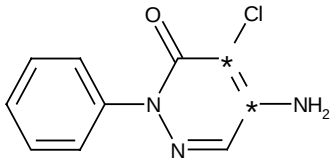
B-7: Residue data

B.7 Residue data

B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1; Annex IIIA 8.1)

SUGAR BEET

The metabolism and distribution of chloridazon in sugar beet was investigated using 5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone-[4, 5-^{14}C]



* Position of ^{14}C labels

Report: Hofmann M., 1990 (a,b,c,d)
Plant uptake study with ^{14}C-chloridazon in sugar beets (pre-emergence)
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany
Fed. Rep., unpublished

Mayer F., 1991
The metabolism of ^{14}C-chloridazon in sugar beets
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany
Fed. Rep., unpublished

GLP: yes

Guideline: not specified

Deviations: none

Acceptability: The study is considered to be acceptable.

Material and Methods:
The metabolism of chloridazon in sugar beet was investigated in four different use patterns. Therefore, four different plant up-take studies were performed in order to obtain sugar beet samples treated with ^{14}C-chloridazon under different conditions. An overview on the different test designs is given in Table B.7.1-1.
In the forth plant up-take study (d) the plants were treated once with 1.95 kg as/ha. The application was done 35 days before harvest in order to create high residues for metabolite identification. Samples of sugar beet leaves were taken 3 hours after the application. Sugar beets, leaves, roots and soil were sampled at harvest time, which was 35 days thereafter.

Table B.7.1-1: Design of plant uptake studies

Study	Hofmann (a)	Hofmann (b)	Hofmann (c)	Hofmann (d)
Intended application rate [kg as/ha]	2.6	5	1.95	1.95
Treatment	Pre	Pre	Post	Post
Soil type	Sand/loam/peat	US soil	Sand/loam/ peat	Sand/loam/peat
Number of applications	1	1	1	1
Comparison to the maximum recommended use rate	1 x	1.9 x	0.75 x	0.75 x
Sampling of unripe material [days after treatment]	62, 80, 112	44, 64	22, 49	0
PHI [days after last application]	190	140	126	35

The ripe samples were separated into roots and tops and extracted with cold methanol. After evaporation to the aqueous phase, the extracts were subsequently partitioned into different organic solvents such as hexane, dichloromethane and ethyl acetate. The extracts were analysed by HPLC and TLC. Metabolites were isolated and identified by HPLC-MS and GC-MS. Methanol unextractable residues were further treated under more vigorous conditions such as soxhlet, acid, base and enzymatic extractions with subsequent HPLC analysis. As radioactivity could be associated with natural components, fractionations for starch, cellulose, lignin and protein were carried out. The radioactivity in the Total Dietary Fiber fractions was determined.
At harvest of the ripe sample material, soil samples were taken additionally. After determination of the total radioactive residue by combustion analysis, the samples were extracted with methanol and methanolic HCl. The extracts were combined and analysed by HPLC.

Findings:
The total radioactive residues (TRR) for the different use patterns were calculated from extractable and residual radioactivity (see Table B.7.1-2) since the results obtained by direct combustion analysis showed a high variability.
Except for the unrealistic late treatment, the total radioactive residue levels in the roots were < 0.1 mg/kg.

Table B.7.1-2: Total radioactive residues (TRR) in sugar beet tops and roots at harvest

Treatment	Application rate (kg as/ha)	Days after application	Sample material	TRR [1] (mg/kg)
Pre-emergence (regular soil)	2.6	189	top	9.578
			root	0.094
Pre-emergence (US soil)	5.0	140	top	0.572
			root	0.069
Post-emergence	1.95	126	top	7.085
			root	0.035
Post-emergence, late treatment	1.95	35	top	46.999
			root	0.296

[1]) TRR = ERR + RRR

From sugar beet tops, 89 – 95 % of the residue was extractable into cold methanol. The partition experiments performed with the methanol extract of tops indicated that higher amounts of radioactivity were organo-soluble. In case of the post emergence samples, more than 80 % of

the TRR was found in the relevant organic phases whereas about 50 % of the TRR were detected for the pre-emergence samples.
79 – 93 % of TRR in the methanol extract could be identified, the rest characterised (see Table B.7.1-3).

Table B.7.1-3: Extractability, composition and partition behaviour of extractable radioactivity in sugar beet tops

	mg/kg (% of TRR)			
	Pre-emergence	**Pre-emergence (US soil)**	**Post-emergence**	**Post-emergence, late treatment**
TRR [1)]	9.578 (100)	0.572 (100)	7.085 (100)	46.999 (100)
ERR	8.525 (89.0)	0.543 (94.9)	6.697 (94.5)	44.265 (94.2)
RRR	1.053 (11.0)	0.029 (5.1)	0.388 (5.5)	2.734 (5.8)
ERR (HPLC)	8.525 (89.0)	0.543 (94.9)	6.697 (94.5)	44.265 (94.2)
Identified:				
Chloridazon	- [3)]	0.020 (3.5)	5.257 (74.2)	41.786 (88.9)
Metabolite A	-	0.388 (67.8)	0.422 (6.0)	1.992 (4.2)
Metabolite B	5.899 (61.6)	0.071 (12.4)	0.804 (11.3)	-
Metabolite B glucoside	0.512 (5.3)	-	0.074 (1.0)	-
Metabolite B conjugate (HPLC - Zone 1B)	0.639 (6.7)	-	-	-
Metabolite B conjugate (HPLC - Zone 1A)	0.529 (5.5)	-	-	-
Total identified:	7.579 (79.1)	0.479 (83.7)	6.557 (92.5)	43.778 (93.1)
Total characterised:	0.947 (9.8)	0.064 (11.2)	0.147 (2.1)	0.487 (1.0)
ERR (partition)	8.776 (91.6)	0.543 (94.9)	6.697 (94.5)	44.265 (94.2)
Hexane phase	0.026 (0.3)	-	0.037 (0.5)	0.354 (0.8)
Dichloromethane phase	0.516 (5.4)	-	5.123 (72.3)	35.581 (75.7)
Ethyl acetate phase	4.388 (45.8)	0.272 (47.5)	0.474 (6.7)	3.296 (7.0)
CO_2-trap (fermentation)	-	0.010 (1.8)	-	-
Ethyl acetate phase [2)]	-	0.025 (4.4)	-	-
Condensed water	-	0.022 (0.4)	-	-
Aqueous phase	4.293 (44.8)	0.227 (39.8)	1.047 (14.8)	3.484 (7.4)

[1)] TRR = ERR + RRR
[2)] Partition after saccharose cleavage and fermentation
[3)] - = not detected

From sugar beet roots, in all trials except for the pre-emergence treatment in the high organic carbon soil, 64 – 81 % of the residue were extractable into cold methanol. In these trials, 55 – 72 % of TRR in the methanol extract could be identified, the rest characterised (see Table B.7.1-4).
Though in different amounts, Metabolite B was in all trials the main portion of the identified compounds present in the methanol extract followed by its glucoside and conjugate. The parent compound chloridazon and its glucoside (Metabolite A) were predominant at the late post-emergence treatment. The structures of these metabolites were confirmed by mass spectrometry (MS).

Table B.7.1-4: Extractability and composition of extractable radioactivity in sugar beet roots

	mg/kg (% of TRR)			
	Pre-emergence	**Pre-emergence (US soil)**	**Post-emergence**	**Post-emergence, late treatment**
TRR [1)]	0.094 (100)	0.069 (100)	0.035 (100)	0.296 (100)
ERR	0.076 (81.3)	0.035 (50.2)	0.023 (64.6)	0.190 (64.3)
RRR	0.017 (18.2)	0.034 (49.3)	0.013 (35.8)	0.105 (35.6)
ERR (HPLC)	0.076 (81.3)	0.035 (50.2)	0.026 (73.5)	0.190 (64.3)
Chloridazon	-[2)]	0.004 (6.3)	0.003 (7.6)	0.109 (36.8)
Metabolite A	-	0.005 (7.6)	0.002 (4.9)	0.014 (4.6)
Metabolite B	0.043 (46.0)	0.008 (11.1)	0.004 (11.6)	0.014 (4.9)
Metabolite B glucoside	0.011 (12.0)	0.003 (4.1)	0.004 (11.3)	0.004 (1.3)
Metabolite B conjugate (HPLC - Zone 1B)	0.009 (9.7)	0.005 (7.6)	0.004 (12.4	0.016 (5.3)
Metabolite B conjugate (HPLC - Zone 1A)	0.004 (4.2)	0.001 (1.7)	0.006 (15.7)	0.007 (2.3)
Total identified:	0.067 (71.9)	0.026 (38.4)	0.023 (63.5)	0.164 (55.2)
Total characterised:	0.009 (9.4)	0.009 (11.9)	0.003 (9.9)	0.028 (9.4)

[1)] TRR = ERR + RRR
[2)] - = not detected

The residual radioactivity residues (RRR) remaining after methanol extraction were further characterised by subsequent soxhlet, acid, base and enzymatic extractions. In the following section, a more detailed description of roots (pre-emergence, 1 x) is given. Similar work-ups were performed with the unextractable residues of the three other trials; the results are summarised in Table B.7.1-5.

The RRR of roots obtained after pre-emergence treatment (1 x rate) was further subjected to soxhlet extraction with methanol and water. Further 1.6 % of the TRR were extracted with methanol, 0.6 % thereof could be partitioned into ethyl acetate and identified mainly as metabolite A and B. 1.8 % of TRR were extracted with water, but only less than 0.2 % could be partitioned into ethyl acetate. The residue after soxhlet extraction was first refluxed with HCl, then with NaOH: The acidic way yielded 0.009 mg/kg (9.9 %) and the basic one 0.011 mg/kg (12.0 %) of radioactivity. Finally, 0.007 mg/kg (treatment with acid) and 0.005 mg/kg (treatment with base) were not extractable.

As radioactive residues could be incorporated into natural components and therefore be difficult to extract, attempts were made in parallel to analyse starch, cellulose, lignin and protein in order to get information about the nature of the non-extractable residues. However, in this case it was found out that only minor portions of the radioactive residue were incorporated in these components. Additionally, for the analysis of the unextractable residue, an enzymatic cleavage using a mixture of cellulase, α,β-amylase and pectinase was performed. Also here, a metabolite pattern, identical with that one of the methanol extract, was obtained. Finally, the methanol unextractable residue was characterised by determining the "Total Dietary Fiber" which comprises the complex organic compounds not digested by human digestive enzymes. After the procedure, which consists of a number of enzymatic cleavage steps, it was found out that 0.006 mg/kg (6.7 % of TRR) were strongly found to the indigestible dietary fiber.

Table B.7.1-5: Extractability and composition of non-extractable radioactivity in sugar beet roots

	mg/kg (% of TRR)		
	Pre-emergence (US soil)	**Post-emergence**	**Post-emergence, late treatment**
Methanol unextractable	0.034 (49.3)	0.013 (35.8)	0.135 (35.6)
Vigorously extractable (Soxhlet + HCl [1])	0.017 (20.9)	0.007 (18.6)	0.006 (2.1)
Identified: Chloridazon	0.002 (2.9)	< 0.001 (1.9)	<0.001 (0.2)
Metabolite A	0.003 (4.1)	0.002 (5.2)	0.006 (1.9)
Metabolite B	0.004 (5.9)	0.002 (4.0)	0.014 (4.8)
Metabolite B glucoside	-[2]	0.001 (2.5)	0.004 (1.3)
Metabolite B conjugate (HPLC - Zone 1B)	-	-	0.003 (0.1)
Metabolite B conjugate (HPLC - Zone 1A)	-	-	0.001 (0.4)
Total identified:	0.009 (12.9)	0.005 (13.6)	0.026 (8.7)
Total characterised:	0.006 (8.1)	0.002 (5.0)	0.008 (2.9)
Finally unextractable	-	-	0.037 (12.5)
Vigorously unextractable (Soxhlet + HCl + Enzymes)	0.020 (28.4)	<0.001 (0.8)	-
Indigestible fiber	0.030 (43.2)	0.008 (21.8)	0.069 (23.4)
Liberated by digestive enzymes	0.010 (14.3	0.004 (10.7)	0.023 (8.3)

[1]) in case of the US soil also enzymes
[2]) - = not detectable

In Table B.7.1-6 the total amounts of the compounds found in the plant matrices investigated are summarised. The amounts of the metabolites present in the corresponding soil samples were added. The structural formulae of the identified components, which were confirmed by MS investigations are given in Table B.7.1-7. The metabolic pathway of chloridazon in sugar beets is shown in Figure B.7.1-1.

From the results, it can be concluded that nature and quantity of the residue in tops and roots strongly depend on the type of soil and the use pattern. The pre-emergence treatments resulted in higher soil residues than the post-emergence treatments. Regular soil treated pre-emergence converted chloridazon almost quantitatively into metabolite B, which was taken up by roots, mainly translocated to the tops and partly further metabolised to different conjugates. The high organic matter soil retained most of the residue as chloridazon. Dephenylation to metabolite B occurred only to a minor degree, probably because the dephenylating microbial activity was lower in this soil. Consequently, low amounts of chloridazon were slowly taken up by the roots and conjugated by the plant to metabolite A, which was the major metabolite in tops under these conditions.

In the post-emergence treatment trials, chloridazon basically stayed on the tops, depending on the pre harvest interval. Part of it was taken up by the plants and converted to metabolite A. The portion of chloridazon that reached the soil was partly dephenylated to metabolite B, taken up by the plant and partly conjugated, especially in the roots.

These results support the assumption that metabolite B is in fact a soil metabolite, taken up by the plant and then further conjugated whereas metabolite A is a plant metabolite.

Table B.7.1-6: Amounts of chloridazon and its metabolites in sugar beet matrices and soil found from different use patterns

Treatment	Appl. rate (kg as/ ha)	Analytes	Residue in mg/kg (% of TRR)		
			tops	roots	soil
Pre-emergence (1 x)	2.6	Chloridazon	-[2)]	-	0.017 (1.1)
		Metabolite A	-	-	
		Metabolite B	5.899 (61.6)	0.043 (46.0)	1.222 (80.5)
		Polar metabolites[1)]	2.627 (27.3)	0.033 (35.3)	
Pre-emergence US soil	5.0	Chloridazon	0.020 (3.5)	0.004 (6.3)	3.911 (86.5)
		Metabolite A	0.388 (67.8)	0.005 (7.6)	
		Metabolite B	0.071 (12.4)	0.008 (11.1)	0.109 (2.4)
		Polar metabolites[1)]	-	0.013 (18.7)	
Post-emergence	1.95	Chloridazon	5.257 (74.2)	0.003 (7.6)	0.185 (48.4)
		Metabolite A	0.422 (6.0)	0.002 (4.9)	
		Metabolite B	0.804 (11.3)	0.004 (11.6)	0.121 (31.6)
		Polar metabolites[1)]	0.221 (3.1)	0.016 (45.2)	
Post-emergence late treatment	1.95	Chloridazon	41.786 (88.9)	0.109 (36.8)	0.091 (80.7)
		Metabolite A	1.992 (4.2)	0.014 (4.6)	
		Metabolite B	-	0.014 (4.9)	0.013 (11.2)
		Polar metabolites[1)]	-	0.033 (11.0)	

1) The polar metabolites consisted of the metabolite B glucoside and conjugates and further metabolites characterised by TLC/HPLC retention behaviour present in the methanol extract.
2) - = not detected

No additional investigations on the extractability/accountability of the incurred residues according to the residue analytical method are required. The same extraction solvent (methanol) is used in all residue analytical methods developed and applied for plant matrices.

Storage stability investigations of stored sample material and stored extracts were performed during the study over a period of 1 to 1.5 years. For testing the stability of the frozen sample material, it was re-extracted and analysed by radiochemical and chromatographic means. For testing the extract stability, the amounts of radioactivity and the metabolite profiles of extracts stored in freezers and/or refrigerator were compared with the relevant data of freshly prepared extracts. The results clearly indicate that both, stored extracts and stored samples are stable over the entire period of investigation.

Table B.7.1-7: Summary of identified components in sugar beet samples after treatment with 14C-chloridazon

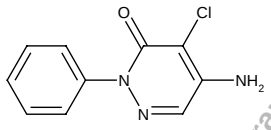
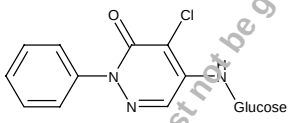
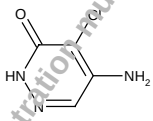
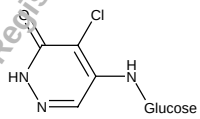
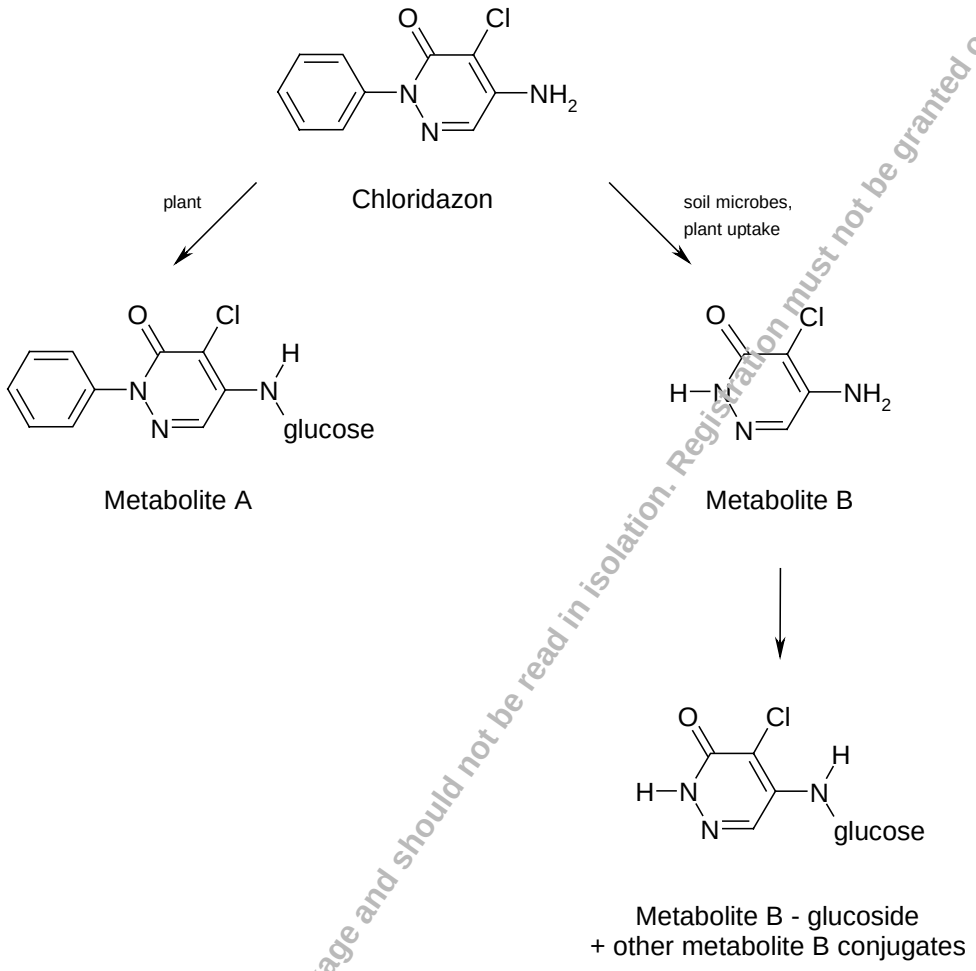
Substance Code (Reg.-No.)	Metabolite identity
Chloridazon (13033)	
Metabolite A (262529)	
Metabolite B (14456)	
Metabolite B-glucose	

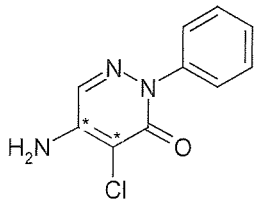
Figure B.7.1-1: Metabolic pattern of chloridazon in sugar beets



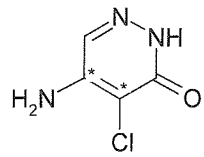
Conclusion:
The metabolism of chloridazon in sugar beets is characterised by the glucosidation of the parent compound and the soil metabolite B. Only minor parts of the total radioactivity were incorporated in starch, cellulose, lignin and protein. The parent compound, the soil metabolite B and the glucosides of both substances form the main residue in sugar beets.

B.7.2 Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2; Annex IIIA 8.1)

The metabolism of chloridazon and of chloridazon metabolite B (the major soil metabolite) in livestock was investigated in lactating goats and laying hens using [pyridazin-4,5-^{14}C]-labelled test items.



Chloridazon



Metabolite B

* denotes position of the ^{14}C-label

METABOLISM OF CHLORIDAZON IN LACTATING GOATS

Reports: Winkler V., Suter P., 1992
Nature of the residue of 14C-BAS 119 H in the lactating goat
[redacted]
Park, NC 27709, USA

Zehr R.D., 1996(c)
Accountability of BASF Method no. D9407 in goat milk
BASF Corporation Agricultural Products Center, Research Triangle Park, NC 27709, USA

GLP: yes

Guideline: EPA 171-4(D)

Deviations: none

Acceptability: The studies are considered to be acceptable.

Material and Methods:
The metabolism and distribution of chloridazon was investigated in two lactating goats following repeated oral administration of ^{14}C-chloridazon at two dose levels. The test item was ^{14}C-labelled at the pyridazin moiety since this position was considered metabolically stable. ^{14}C-chloridazon was administered daily on 7 consecutive days at dose levels of 1 mg/kg feed (low dose) and 66.7 mg/kg feed (high dose). The test item was dissolved in methanol and administered in capsules using cellulose as carrier. Two control animals were dosed with blank capsules containing the respective amount of methanol and cellulose. Details of the study outline are summarised in Table B.7.2-1:

Table B.7.2-1: Dosing of lactating goats with ^{14}C-chloridazon

Dose group	Number of animals	Treatment days	Nominal daily dose	Actual daily dose		Sacrifice time
			mg/kg feed intake	mg/kg feed intake	mg/animal/day	Hours post last dose
Low dose	1	7	1	1.042	1.67	< 24
High dose	1	7	66.7	66.739	109.19	< 24

Sampling and sample storage
Milk was sampled twice daily, in the morning (before administration of the test item) and in the afternoon. Morning milk was pooled with the afternoon milk of the previous day to give a composite sample. Urine and feces were collected once a day in the morning.
The animals were sacrificed within 24 hours upon the last administration and liver, kidney, muscle, fat, bile, blood and heart of the low and high dose goat and the gastrointestinal tract including contents (only high dose goat) were taken for determination of radioactivity. All samples were kept frozen at -20 °C.

Measurement of radioactivity
Aliquots of liquid samples (milk, urine) were directly analysed after mixing with scintillation cocktail without additional treatment. Radioactivity in feces and all non-fatty tissues (including blood) was determined by combustion. Fat samples were treated with tissue solubiliser prior to LSC measurement.

Extraction
Milk, urine and edible tissues from the high dose group were further processed in order to characterise and/or identify relevant metabolites. Milk and tissues were extracted with methanol and the methanol extracts were investigated by radio-HPLC analysis. Urine was directly submitted to HPLC analysis after filtration. Non-extractable residues in muscle, liver, kidney and fat were treated with a non-specific protease and in the case of liver the solubilised bound residue was further subjected to an acid hydrolysis.

Metabolite identification
Goat urine and milk from the high dose animal was used to isolate, purify, and identify the major metabolites by GC-MS, API-LC-MS and by ^{1}H-NMR to specify the location of metab-olically inserted functional groups. These isolated metabolites were used as reference compounds for the identification of metabolites by means of co-chromatography with extracts of muscle, liver and kidney.

Findings:
Excretion, retention and recovery of radioactivity
Following administration of ^{14}C-chloridazon to lactating goats, the radioactivity was rapidly excreted (see Table B.7.2-2). The total recoveries of radioactivity were 91 % in the low dose experiment and 93 % in the high dose experiment. Radioactivity in milk reached a plateau after approximately two days and amounted to 1.4 % and 1.1 % of the low and high dose, respectively.

Table B.7.2-2: Material balance after administration of ^{14}C-chloridazon to lactating goats

Matrix	Material balance in % of total dose	
	Low dose	High dose
Organs and tissues		
Muscle	0.231	0.452
Fat	0.017	0.039
Liver	0.222	0.269
Kidney	0.009	0.013
Heart	0.003	0.008
Milk	1.39	1.13
Blood	0.043	0.068
Upper GI Tract *	n.d.	0.030
Upper GI Tract contents	n.d.	0.177
Lower GI Tract	n.d.	0.927
Urine	61.21	66.95
Feces	27.59	23.21
Total	90.72	93.27

n.d. = not determined
*) GI tract = gastro-intestinal tract

Total radioactive residues

In the low dose goat, total radioactive residues were very low in milk and all tissues, ranging from 0.0003 mg/kg to 0.0097 mg/kg. Only in the liver higher residues were determined with 0.0378 mg/kg. Total radioactive residues in tissues of the high dose animal were also lowest in fat and muscle, followed by kidney and milk. Again, highest residues were found in the liver with 2.034 mg/kg. Table B.7.2-3 gives a summary of the individual TRR values for each matrix by dose group.

Table B.7.2-3: Total radioactive residues in edible matrices after dosing of lactating goats with ^{14}C-chloridazon

Matrix	Dose group: 1 mg/kg feed [mg/kg]	Dose group: 66.7 mg/kg feed [mg/kg]
Milk (Day 7)	0.0097	1.066
Muscle	0.0018	0.194
Fat	0.0003	0.051
Liver	0.0378	2.034
Kidney	0.0060	0.679

All results are calculated as parent equivalents.

Extractability

The extractability of edible matrices of the high dose goat with methanol and water is presented in Table B.7.2-4. Samples of the low dose goat were not extracted due to the very low residue levels.

Table B.7.2-4: Extractability of edible matrices after dosing of lactating goats with ^{14}C-chloridazon at a nominal dose level of 66.7 mg/kg feed

Matrix	TRR	Methanol	Water	ERR	RRR	Recovery
	mg/kg	mg/kg (% TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)
Milk (Day 7)	1.066	1.092 (102.4)	n. p.	1.092 (102.4)	0.014 (1.3)	1.106 (103.7)
Muscle	0.194	0.155 (80.0)	0.003 (1.4)	0.158 (81.4)	0.040 (20.5)	0.198 (101.9)
Fat	0.051	0.039 (76.5)	n. p.	0.039 (76.5)	0.010 (20.0)	0.049 (96.5)
Liver	2.034	0.458 (22.5)	0.071 (3.5)	0.529 (26.0)	1.541 (75.9)	2.070 (101.9)
Kidney	0.679	0.514 (75.6)	0.018 (2.6)	0.532 (78.2)	0.154 (22.7)	0.686 (100.9)

n. p. not performed
TRR: Total radioactive residue
ERR: Extractable radioactive residue
RRR: Residual (= non-released) radioactive residue

Identification and characterisation of residues

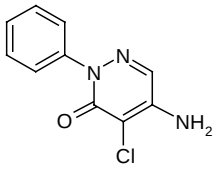
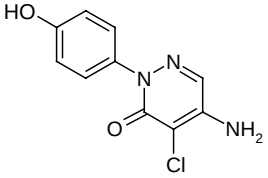
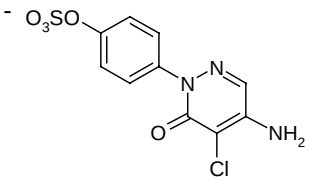
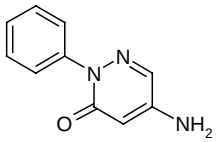
As mentioned above, only the tissues and milk of the high dose group were extracted and used for metabolite identification.

Major metabolites (parent compound chloridazon, 4-hydroxy-chloridazon, dechlorinated chloridazon) were isolated from goat urine and milk from the high dose group and identified by GC-MS and/or API-LC-MS. The structure of metabolite *4*-hydroxy chloridazon (i.e. chloridazon hydroxylated at the phenyl ring in *4*-position to the attached pyridazin moiety) was further investigated by ^{1}H-NMR to specify the location of the metabolically inserted hydroxyl group.

These isolated metabolites were used as reference substances for the identification of metabolites in extracts of milk, muscle, liver, fat and kidney by means of co-chromatography. A summary of all identified metabolites and their distribution in milk and tissues is given in Table B.7.2-5.

The main residue in all investigated samples was dechlorinated chloridazon except for the milk, where the sulfate conjugate of the 4-hydroxylated chloridazon was the major metabolite. Unchanged parent compound was found in milk and all tissues, representing a main residue in milk, fat and muscle while playing a subordinate role in the kidney and liver.

Table B.7.2-5: Summary of identified and quantified metabolites in edible matrices of lactating goats after dosing with ^{14}C-chloridazon at a nominal dose level of 66.7 mg/kg (based on feed intake)

Metabolite Code [1)]	Structure	Milk [2)]	Muscle	Fat	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Chloridazon (BAS 119 H)		0.338 (31.7)	0.021 (10.9)	0.012 (22.5)	0.117 [2)] (5.7)	0.036 (5.3)
4-OH-Chloridazon (BH 119-4-OH)		0.212 (19.9)	0.011 (5.4)	0.004 (8.1)	0.089 (4.4)	0.118 (17.4)
Sulfate conjugate of BH 119-4-OH		0.423 (39.7)	n.d.	n.d.	n.d.	n.d
Deschloro-Chloridazon		0.118 (11.1)	0.115 (59.4)	0.018 (36.1)	0.611 [2)] (29.9)	0.144 (21.1)
Total identified		1.091 (102.4)	0.147 (75.7)	0.034 (66.7)	0.817 (40.0)	0.298 (43.8)

1) the codes in brackets are the metabolic designation as they were used in the original report
2) figures represent sum of amounts found in methanol extract and in the protease released fractions
n.d. not detected

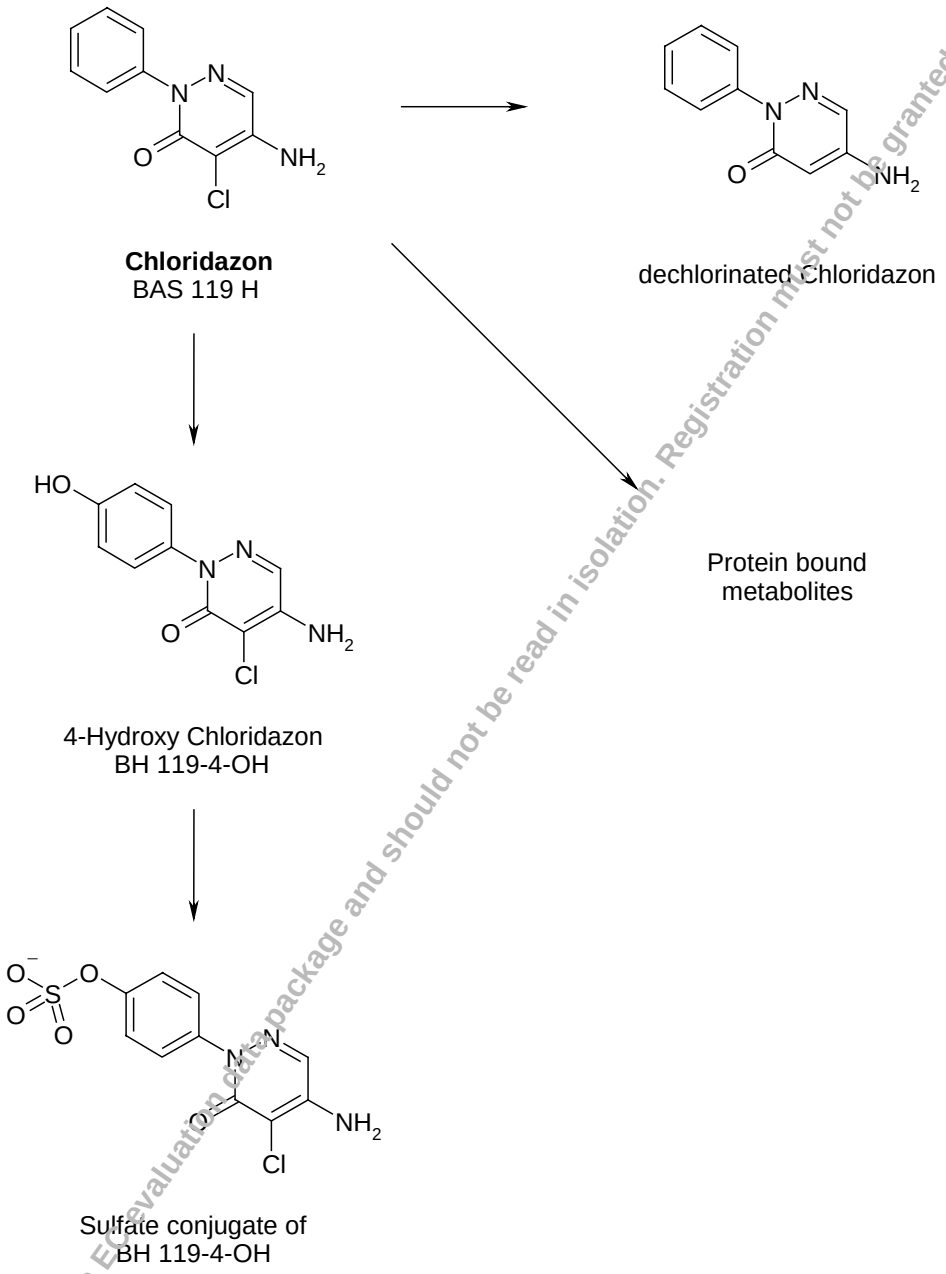
Storage stability

All samples were analysed within 6 months after sampling. To confirm storage stability, the methanol extracts of tissues and milk were reanalysed in the middle of the study (9 to 10 months after receipt of samples) and towards the end of the study (tissues: 20 months after sampling, milk: 14 months after sampling). In milk, fat, liver and muscle the metabolic profiles remained unchanged over the 14-month storage period (milk: 9 months storage). In kidney, the polar sulfate conjugates of 4-hydroxy-chloridazon appeared to have slowly converted to 4-hydroxy chloridazon after storage for 14 months. However, the metabolic profile did not change until the middle of the study (3 months storage) and quantitative analysis was performed within this time frame. Therefore, this conversion did not affect the qualitative and quantitative results of the study.

Metabolic pathway

The metabolic pathway of chloridazon in the goat is depicted in see Figure B.7.2-1. The two main routes of metabolic conversion observed in this study were the dechlorination of the parent compound and its hydroxylation at the phenyl ring in 4-position to the pyridazin moiety, leading to metabolite 4-hydroxy-chloridazon. The hydroxyl compound is then further transformed into the sulfate conjugate via typical phase II metabolic reactions enhancing its hydrophilicity and elimination.

Figure B.7.2-1: Proposed metabolic pathway of chloridazon in the goat



METABOLISM OF CHLORIDAZON METABOLITE B IN LACTATING GOATS

Reports: Leibold E., Hoffmann H.D., 1999(a)
14-C-Metabolite B of chloridazon - Absorption distribution and excretion after repeated oral administration in lactating goats
[redacted]

Kampke-Thiel K., 1999(a)
The metabolism of ^{14}C-metabolite B (Reg. No. 014 456) of chloridazon in lactating goat
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep.

GLP: yes

Guideline: EPA 860.1300

Deviations: none

Acceptability: The studies are considered to be acceptable.

Material and Methods:
The metabolism and distribution of ^{14}C-chloridazon metabolite B was investigated in two lactating goats following repeated oral administration at one dose level. The test item was ^{14}C-labelled at the pyridazin moiety since this position was considered metabolically stable. ^{14}C-metabolite B was administered to two goats daily on 5 consecutive days at a nominal dose level of 12 mg/kg feed. The test item was dissolved in a 0.5 % aqueous Tylose solution and administered by gavage with a syringe connected to an intubation catheter through the mouth of the animals. Details of the study outline are summarised in Table B.7.2-6.

Table B.7.2-6: Dosing of lactating goats with ^{14}C-metabolite B of chloridazon

Animal No.	Treatment days	Nominal daily dose	Actual daily dose		Sacrifice time
		mg/kg feed intake	mg/kg feed intake	mg/kg bw	Hours post last dose
Goat 1	5	12	11.22	0.65	23
Goat 2	5	12	13.15	0.50	23

Sampling
Urine and feces were collected in time intervals of 24 hours. Milk was sampled twice a day, in the morning before administration of the test substance and in the afternoon.
In order to get some information on the blood and plasma concentration of radioactivity, blood samples were taken 1 hour before and 1 hour after administration during the application period and on the last day of application additionally at 2, 3, 4, 6, 8, and 23 hours post dosing. 23 hours after the last administration, animals were sacrificed and liver, kidneys, blood, kidney and intraperitoneal fat, back and leg muscles, bile, urine in bladder and the gastrointestinal tract plus contents were taken for determination of radioactivity.
Prior to metabolite investigations tissue and milk samples of the two individual animals were pooled.

Measurement of radioactivity

Aliquots of liquid samples (milk, urine, plasma, bile, cage wash) were directly analysed after mixing with scintillation cocktail without additional treatment. Feces and the GI tract (+contents) were treated with tissue solubiliser after freeze-drying. Blood samples were bleached before mixing with scintillation cocktail. Radioactivity in tissues was determined by combustion.

Extraction and analysis

Muscle, liver and kidney samples were extracted with methanol and the extracts further purified by an iso-hexane partition. Milk and fat were extracted with a 1:1 mixture of methanol/iso-hexane. In the case of milk, a protein precipitation by addition of acetone was performed with the methanol extract. The extraction residue of fat was further treated with methanol in a microwave oven at 150 °C.

The methanol extracts of milk and tissues were investigated by radio-HPLC and metabolites were identified by co-chromatography with synthetic reference substances on two different HPLC systems.

Findings:

Excretion, retention and recovery of radioactivity

Following administration of ^{14}C-metabolite B of chloridazon to lactating goats, the radioactivity was rapidly excreted (see Table B.7.2-7).

Generally, the concentration of radioactivity in afternoon milk was considerably higher than in the morning milk, increasing during days 1 to 4 and on day 5 slightly declining (Goat 1) or remaining virtually constant (Goat 2). In both animals, the time course of milk concentration was rather similar to plasma concentration. Radioactivity in milk amounted to 2.7 % and 1.8 % of the dose.

Table B.7.2-7: Material balance after administration of ^{14}C-metabolite B of chloridazon to lactating goats

Matrix	Material balance in % of total administered dose	
	Goat 1	Goat 2
Organs and Tissues		
Liver	0.13	0.16
Kidney	0.03	0.03
Muscle*	0.70	0.72
Fat	0.02	0.04
Milk	2.73	1.76
GI Tract**	2.16	5.31
Blood	0.11	0.27
Bile	0.00	0.00
Urine in bladder	0.11	--
Urine	87.53	77.23
Feces	7.31	8.59
Cage wash	1.86	3.87
Total	102.67	98.01

*) Sum of back and leg muscle samples
**) Sum of gut, gut content, stomach, and stomach content

Total radioactive residues

The total radioactive residues in milk and the tissues destined for human consumption, which are summarised in Table B.7.2-8, were all very similar, ranging from 0.182 mg/kg to 0.272 mg/kg, with the lowest value in muscle and the highest residue in kidney.

Table B.7.2-8: Total radioactive residues in edible matrices after dosing of lactating goats with ^{14}C-metabolite B of chloridazon at a nominal dose level of 12 mg/kg feed

Matrix	TRR [mg/kg]
Milk (Pool of Days 2-5)	0.267
Muscle	0.182
Fat	0.214
Liver	0.191
Kidney	0.272

Extractability

The extractabilities of milk and all tissues with methanol ranged from 82 to 106 % TRR being recovered from the organic extracts (see Table B.7.2-9). As could be assumed from the polar nature of the test item, virtually no radioactivity went into hexane, when extracting fat and milk with a 1:1 mixture of methanol/iso-hexane. In the case of fat, the non-extractable residues were further treated with a microwave-assisted methanol extraction, which released 94 % of the bound radioactivity.

Table B.7.2-9: Extractability of edible matrices after dosing of lactating goats with ^{14}C-metabolite B of chloridazon at a nominal dose level of 12 mg/kg feed

Matrix	TRR	Methanol	Iso-Hexane	ERR	RRR	Recovery
	mg/kg	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Milk (Pool of days 2-5)	0.267	0.260 (97.7)	0.000 (0.0)	0.260 (97.7)	--[1)]	0.260 (97.7)
Muscle	0.182	0.180 (98.7)	n.p.	0.180 (98.7)	0.008 (4.6)	0.188 (103.3)
Fat	0.214	0.175 (82.0)	0.000 (0.1)	0.175 (82.1)	0.034 (15.8)	0.209 (97.9)
Liver	0.191	0.185 (97.0)	n.p.	0.185 (97.0)	0.009 (4.9)	0.194 (101.9)
Kidney	0.272	0.287 (105.6)	n.p.	0.287 (105.6)	0.007 (2.5)	0.294 (108.1)

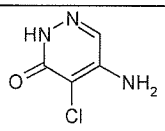
[1)] no solid residue
n.p.: not performed
TRR: Total radioactive residue
ERR: Extractable radioactive residue
RRR: Residual (= non-released) radioactive residue

Identification and characterisation of residues

HPLC investigations were performed with the methanol extracts of milk, muscle, fat, kidney and liver after partition with iso-hexane or acetone precipitation (milk). The obtained metabolite patterns of all samples showed one single, very polar peak, which was identified as unchanged chloridazon metabolite B by co-chromatography with the reference compound on two different HPLC systems. Similarly, the bound radioactivity in the fat, which was released into methanol by

a microwave-assisted extraction at 150 °C consisted of the same single peak identified as metabolite B.
The quantitative results are summarised in Table B.7.2-10.

Table B.7.2-10: Summary of identified and quantified metabolites in edible matrices of lactating goats after dosing with ^{14}C-metabolite B of chloridazon at a nominal dose level of 12 mg/kg (based on feed intake)

Metabolite Code	Structure	Milk	Muscle	Fat	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Chloridazon metabolite B (Reg. No. 14456)		0.255 (95.8)	0.173 (94.6)	0.207[1)] (97.0)	0.186 (97.4)	0.292 (107.4)

[1)] represents sum of amounts found in the extract and the solubilised bound residues

Storage stability
All samples were extracted and analysed within one month after sampling.

METABOLISM OF CHLORIDAZON IN LAYING HENS

Report: Wu D., 1991
Nature of the residue of 14C-BAS 119 H in the laying hen
[redacted]
Park, NC 27709, USA

GLP: yes

Guideline: EPA 171-4

Deviations: none

Acceptability: The study is considered to be acceptable.

Material and Methods:
The metabolism and distribution of chloridazon was investigated in laying hens following repeated oral administration of ^{14}C-chloridazon at two dose levels. The test compound was ^{14}C-labelled at the pyridazin moiety since this position was considered metabolically stable. ^{14}C-chloridazon was administered daily on 10 consecutive days at dose levels of 0.1 mg/kg feed (low dose) and 10 mg/kg feed (high dose). The test item was dissolved in methanol and administered in capsules using cellulose as carrier. A control group was dosed with blank capsules containing the respective amount of methanol and cellulose. Details of the study outline are summarised in Table B.7.2-11.

Table B.7.2-11: Dosing of laying hens with ^{14}C-chloridazon

Dose group	Number of animals	Treatment days	Nominal daily dose	Actual daily dose		Sacrifice time
			mg/kg feed intake	mg/kg feed intake	mg/animal/day	Hours post last dose
Low dose	10	10	0.1	0.09	0.0097	<24
High dose	10	10	10	8.97	0.942	<24

Sampling and sample storage

Eggs were collected twice a day, in the morning (before administration of the test substance) and in the afternoon. The eggs were pooled as whole eggs (minus shell) each day by treatment group. Excreta were collected once a day in the morning. Blood was drawn prior to necropsy. The animals were sacrificed within 24 hours upon the last administration and skin, breast and thigh muscle, liver, heart, fat, gizzard (without contents), kidneys and leg bones were taken for determination of radioactivity. Each sample type was pooled by treatment group. All samples were kept frozen at -20 °C.

Measurement of radioactivity

Radioactivity in excreta, eggs and all non-fatty tissues was determined by combustion. Fat and skin samples were treated with tissue solubiliser before counting.

Extraction

Since the residues in all samples of the low dose group and in muscle and fat of the high dose animals were below 0.01 mg/kg, only excreta, eggs and liver samples from the high dose group were further processed in order to characterise and/or identify relevant metabolites. Samples were extracted with methanol (and in the case of liver subsequently with water) and the methanol extracts were investigated by radio-HPLC analysis. Non-extractable residues in liver and egg were treated with a non-specific protease and the solubilised bound residues were further subjected to an acid hydrolysis.

Metabolite identification

The metabolite identification was performed by co-chromatography with reference compounds on HPLC and TLC, also using metabolites isolated from goat urine of a separate metabolism study with chloridazon in goats [see 1992/5162 Winkler V., Suter P., 1992], the structures of which were elucidated by GC-MS, API-LC-MS and by ^{1}H-NMR.

Findings:

Excretion, retention and recovery of radioactivity

Following administration of ^{14}C-chloridazon to laying hens, the radioactivity was rapidly excreted (see Table B.7.2-12), with excreta containing 87 % to 92 % of the dose. Radioactivity in eggs reached a plateau after approximately 8 days and amounted to 0.1 % and 0.2 % of the dose in the low and high dose group, respectively. No indication of accumulation of chloridazon in poultry tissues was found.

Table B.7.2-12: Material balance after administration of ^{14}C-chloridazon to laying hens

Matrix	Material balance in % of total administered dose	
	Low dose	High dose
Organs and tissues		
Muscle (breast+thigh)	0	0.01
Fat	0	0.001
Liver	0.1	0.047
Kidney	0	0.003
Skin	0	0.002
Heart	0	0.001
Whole Eggs	0.1	0.2
Eggshell	0.00	0.014
Blood	0	0.004
Gizzard	0	0.008
Bones	0	0.003
Excreta	86.9	92.3
Total	87.1	92.6

Total radioactive residues

In the tissues of animals of the low dose group, total radioactive residues were generally very low and especially in muscle and fat they were below the limit of detection. Tissues of animals that received the dose of 10 mg/kg feed showed as well low total radioactive residues, which ranged from 0.0033 mg/kg in fat up to 0.1952 mg/kg in liver. Table B.7.2-13 gives a summary of the individual TRR values for each matrix by dose group.

Table B.7.2-13: Total radioactive residues in edible matrices after dosing of laying hens with ^{14}C-chloridazon

Matrix	Dose group: 0.1 mg/kg feed [mg/kg]	Dose group: 10 mg/kg feed [mg/kg]
Whole Egg (Day 10)	0.00042	0.0584
Breast Muscle	<LOD *	0.0054
Thigh Muscle	<LOD *	0.0065
Fat	<LOD *	0.0033
Liver	0.00344	0.1952

All results are calculated as parent equivalents.

*) The Limit of Detection (LOD) was individually determined for each tissue type: Breast muscle 8.406 x 10^{-5} mg/kg, thigh muscle 8.133 x 10^{-5} mg/kg, fat 8.563 x 10^{-5} mg/kg.

Extractability

The extractability of eggs and liver of the high dose animals with methanol and water is presented in Table B.7.2-14. Samples of the low dose group as well as fat and muscle tissue of the high dose animals were not extracted due to the very low residue levels which were below 0.01 mg/kg and thus are exempt from further investigation.

The non-extractable residues of liver and egg were subjected to an enzymatic treatment with pronase, which resulted in a further release of the bound radioactivity.

Table B.7.2-14: Extractability of edible matrices after dosing of laying hens with ^{14}C-chloridazon at a nominal dose level of 10 mg/kg feed

Matrix	TRR	Methanol	Water	ERR	RRR	Recovery
	mg/kg	mg/kg (% TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)
Whole Egg	0.0584	0.031 (53.2)	n.p.	0.031 (53.2)	0.027 (46.0)	0.058 (99.2)
Liver	0.1952	0.023 (11.8)	0.010 (5.1)	0.033 (16.9)	0.165 (85.2)	0.198 (102.1)

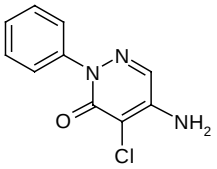
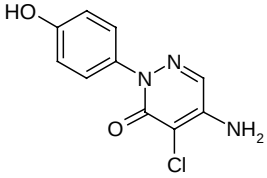
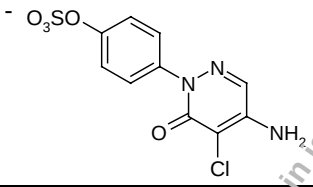
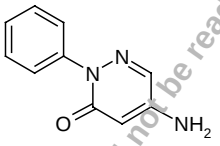
n. p. not performed
TRR: Total radioactive residue
ERR: Extractable radioactive residue
RRR: Residual (= non-released) radioactive residue

Identification and characterisation of residues

Due to the lack of sufficient amount of material for structural identification, hen metabolites were not isolated but mostly identified by co-chromatography (HPLC, TLC) with metabolites (BH 119-4-OH, dechlorinated BAS 119 H) isolated from goat urine and milk of a separate metabolism study, the structures of which were elucidated by GC-MS, ^{1}H-NMR and/or API-LC-MS [see 1992/5162 Winkler V., Suter P., 1992]. One metabolite in eggs was isolated and identified by GC after methylation.

Identified metabolites of hen eggs and liver are summarised in Table B.7.2-15.

Table B.7.2-15: Summary of identified and quantified metabolites in edible matrices of laying hens after dosing with ^{14}C-chloridazon at a nominal dose level of 10 mg/kg (based on feed intake)

Metabolite Code [1)]	Structure	Egg	Liver
		mg/kg (% TRR)	mg/kg (% TRR)
Chloridazon (BAS 119 H)		n.d.	0.053 [2)] (27.1)
BH 119-4-OH (4-hydroxy chloridazon)		0.013 (22.0)	n.d.
BH 119-4-OH sulfate (Sulfate conjugate of 4-OH chloridazon)		0.010 (17.0)	n.d.
Dechlorinated chloridazon (deschloro chloridazon)		0.008 (14.2)	n.d
Total identified		0.031 (53.2)	0.053 (27.1)

1) the codes in brackets are the metabolic designation as used in the original report
2) identified in the protease released fractions
n.d. not detected

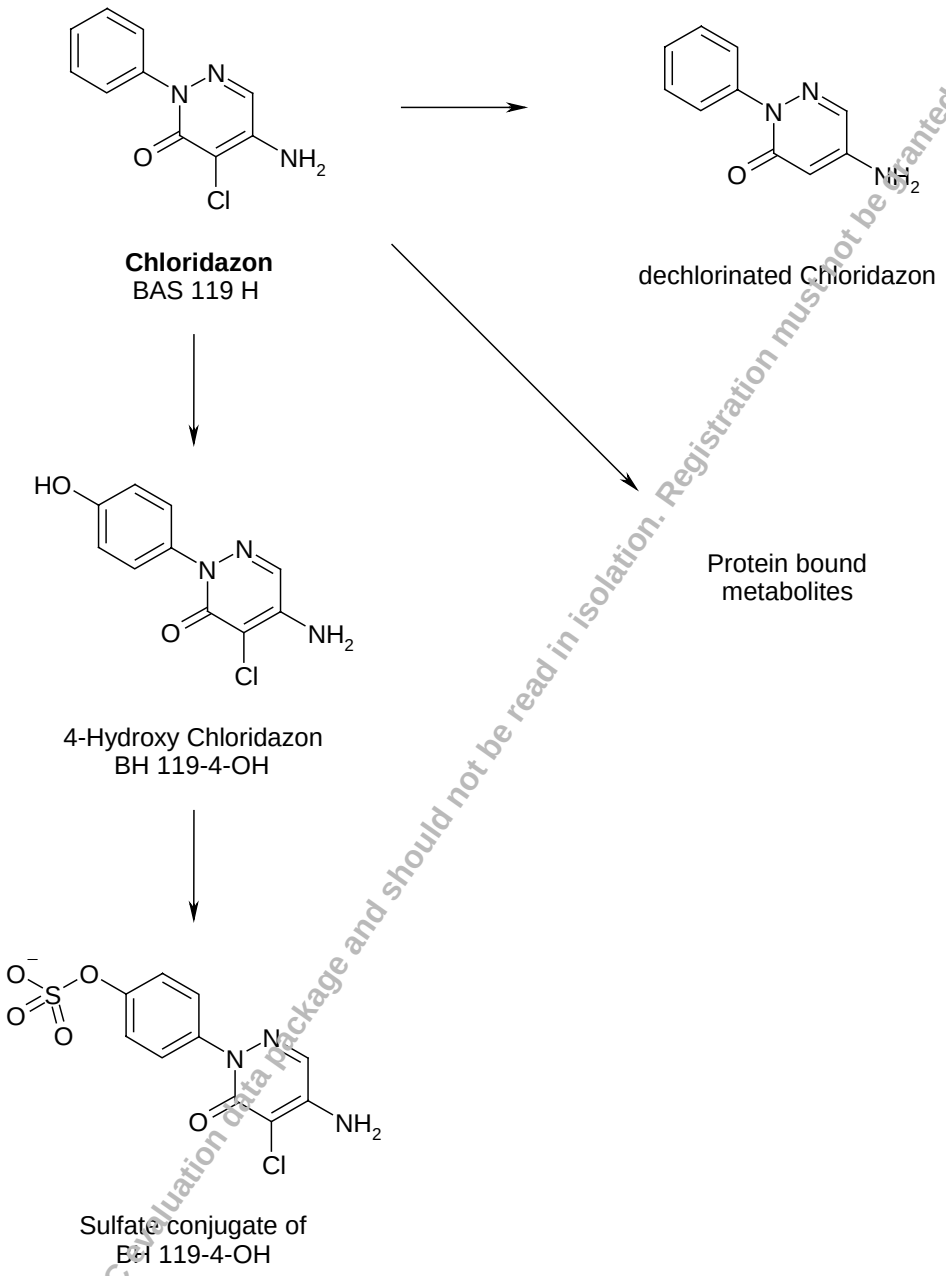
Storage stability

The samples were extracted and analysed within 6 months after receipt. To confirm storage stability, freshly prepared methanol extracts of eggs and liver were analysed at the beginning and towards the end of the study. The metabolic profiles remained unchanged over the 10- and 12-month storage period of liver and eggs, indicating that the samples were stable within this time span.

Metabolic pathway

The metabolic pathway of chloridazon in the hen is depicted in Figure B.7.2-2. The two main routes of metabolic conversion observed in this study were the dechlorination of the parent compound and its hydroxylation in 4-position at the phenyl moiety, leading to metabolite BH 119-4-OH. The hydroxyl compound is then further transformed into the sulfate conjugate via typical phase II metabolic reactions enhancing its hydrophilicity and elimination.

Figure B.7.2-2: Proposed metabolic pathway of chloridazon in hens



METABOLISM OF CHLORIDAZON METABOLITE B IN LAYING HENS

Reports: Leibold E., Hoffmann H.D., 1999(b)
14C-Metabolite B of chloridazon - Absorption, distribution and excretion after repeated oral administration in laying hens
[redacted]

Kampke-Thiel K., 1999(b)
The metabolism of ^{14}C-Metabolite B (Reg. No. 014 456) of chloridazon in laying hens
[redacted]

Kampke-Thiel K., Hartl M., 2002
Amendment No. 1: The metabolism of ^{14}C-Metabolite B (Reg. No. 014456) of chloridazon in laying hens
[redacted]

GLP: yes

Guideline: EPA 860.1300

Deviations: none

Acceptability: The studies are considered to be acceptable.

Material and Methods:
The metabolism and distribution of ^{14}C-chloridazon metabolite B was investigated in laying hens following repeated oral administration at one dose level. The test item was ^{14}C-labelled at the pyridazin moiety since this position was considered metabolically stable. ^{14}C-metabolite B was administered to a group of eleven hens daily on 8 consecutive days at a nominal dose level of 12 mg/kg feed. The test item was dissolved in a 0.5 % aqueous Tylose solution and administered orally by gavage with a syringe. Details of the study outline are summarised in Table B.7.2-16:

Table B.7.2-16: Dosing of laying hens with ^{14}C-metabolite B of chloridazon

Dose group	Number of animals	Treatment days	Nominal daily dose	Actual daily dose		Sacrifice time
			mg/kg feed intake	mg/kg feed intake	mg/kg bw	Hours post last dose
Low dose	11	8	12	14	0.91	23

Sampling
Excreta were collected in time intervals of 24 hours. Eggs were sampled twice a day, in the morning before administration of the test substance and in the afternoon.
In order to get some information on the plasma concentration of radioactivity, blood samples were taken on the last day of application at 1 hour before dosing and at 2, 4, 6, 8 and 23 hours post dosing.

23 hours after the last administration, animals were sacrificed and liver, kidneys, blood, adipose tissue, chest and leg muscles, gastrointestinal tract (skin and contents) and the skin were taken for determination of radioactivity.
For metabolite investigations, eggs from day 2 to day 8 as well as each tissue type were pooled over all animals.

Measurement of radioactivity
Aliquots of liquid samples (plasma, cage wash) were mixed with scintillation cocktail and analysed without additional treatment. Excreta and the GI tract (+contents) were treated with tissue solubiliser after freeze-drying. Blood samples were bleached before mixing with scintillation cocktail. Radioactivity in tissues was determined by combustion.

Extraction and analysis
Muscle and liver samples were extracted with methanol and the extracts further purified by an iso-hexane partition. Eggs were extracted with a 1:1 mixture of methanol/iso-hexane and fat and skin samples with acetonitrile/iso-hexane (1:1). The extraction residues of liver and eggs were further treated with methanol in a microwave oven at 150 °C.
The methanol and acetonitrile extracts of eggs and tissues were investigated by radio-HPLC.
Metabolites in the extracts were identified by co-chromatography with synthetic reference substances on two different HPLC systems.

Findings:
Excretion, retention and recovery of radioactivity
Following administration of ^{14}C-metabolite B of chloridazon to laying hens, the radioactivity was rapidly excreted within 24 hours after application (see Table B.7.2-17). Excreta contained 87 % of the dose. Together with cage wash, the total amount of excreted radioactivity was found to be 91.5 % of the administered dose.
No indication of accumulation of chloridazon metabolite B in poultry tissues was found.

Table B.7.2-17: Material balance after administration of ^{14}C-metabolite B of chloridazon to laying hens

Matrix	Material balance in % of total administered dose
Organs and Tissues	
Liver	0.04
Kidney	0.01
Muscle*	0.40
Adipose tissue	0.00
Skin	0.05
Eggs	1.50
GI Tract (skin)	0.08
GI Tract (contents)	0.12
Blood	0.01
Excreta	87.00
Cage wash	4.54
Total	93.74

*) Sum of chest and leg muscle samples (0.18 % and 0.22 % of dose, respectively)

Total radioactive residues
The total radioactive residues in eggs and the tissues destined for human consumption are summarised in Table B.7.2-18. The residues were generally low, ranging from 0.012 mg/kg in fat to 0.506 mg/kg in eggs.

Table B.7.2-18: Total radioactive residues in edible matrices after dosing of laying hens with ^{14}C-metabolite B of chloridazon at a nominal dose level of 12 mg/kg feed

Matrix	TRR [mg/kg]
Egg (Pool of Days 2-8)	0.506
Muscle	0.129
Fat	0.012
Skin	0.087 [1)]
Liver	0.141

[1)] Value represents sum of extractable radioactivity (ERR) and non-extractable residue (RRR) since sample was not suitable for combustion

Extractability

The extractabilities of tissues and eggs with methanol or acetonitrile were ranging between 86 % TRR and 100 % TRR (see Table B.7.2-19).

Table B.7.2-19: Extractability of edible matrices after dosing of laying hens with ^{14}C-metabolite B of chloridazon at a nominal dose level of 12 mg/kg feed

Matrix	TRR	Methanol	Acetonitrile	Iso-Hexane	ERR	RRR	Recovery
	mg/kg	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Egg	0.506	0.434 (85.8)	n.p.	0.003 (0.6)	0.437 (86.4)	0.058 (11.5)	0.495 (97.8)
Muscle	0.129	0.129 (99.8)	n.p.	n.p.	0.129 (99.8)	0.004 (3.0)	0.133 (102.8)
Fat	0.012	n.p.	0.011 (87.6)	0.001 (5.7)	0.012 (93.3)	0.001 (8.2)	0.013 (101.5)
Skin	0.087	n.p.	0.075 (86.2)	0.002 (1.8)	0.077 (88.0)	0.010 (12.0)	0.087 (100.0)
Liver	0.141	0.132 (93.8)	n.p.	n.p.	0.132 (93.8)	0.009 (6.1)	0.141 (99.9)

n.p. not performed
TRR: Total radioactive residue
ERR: Extractable radioactive residue
RRR: Residual (= non-released) radioactive residue

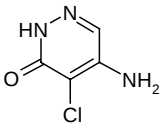
Identification and characterisation of residues

HPLC investigations were performed with the methanol extracts of eggs, muscle and liver (after partition with iso-hexane), with the acetonitrile extracts of fat and skin and with the solubilised bound residue of eggs.

The obtained metabolite patterns of the organic extracts of all samples were identical showing one single, very polar peak, which was identified as unchanged chloridazon metabolite B by co-chromatography with the reference compound on two different HPLC systems. Similarly, the bound radioactivity in eggs, which was released into methanol by a microwave-assisted extraction at 150 °C consisted of the same single peak identified as metabolite B.

The quantitative results are summarised in Table B.7.2-20.

Table B.7.2-20: Summary of identified and quantified metabolites in edible matrices of laying hens after dosing with ^{14}C-metabolite B of chloridazon at a nominal dose level of 12 mg/kg feed

Metabolite Code	Structure	Eggs	Muscle	Fat	Skin	Liver
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
chloridazon Metabolite B (Reg. No. 14456)		0.481[1] (95.1)	0.128 (99.2)	0.011 (87.6)	0.075 (86.2)	0.130 (92.8)

[1]) represents sum of amounts found in the extract and the solubilised bound residues

Storage stability
All samples were extracted and analysed within six months after sampling.

Conclusion:
The metabolic pathway of chloridazon in livestock animal was investigated in lactating goats and laying hens. In both cases the main biotransformations were a dechlorination of the parent substance and a hydroxylation at the phenyl ring. The hydroxylation was followed by a further conjugation with sulfate.
Separately, the metabolism of metabolite B (the main soil metabolite) in animals was investigated. It was shown that this substance did not transform and was found unchanged in all animal matrices. The radioactivity was rapidly excreted within 24 hours after application.

B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6)

B.7.3.1 Residue definition in plant matrices:

Metabolism studies were performed in the crop category 'root crops' with the crop: sugar beet. Based on the facts presented the following definitions of relevant residues are proposed for MRL setting and dietary risk assessment in root crops:

Residue definition for monitoring:	Chloridazon and metabolite B, expressed as chloridazon equivalents
Residue definition for risk assessment:	Chloridazon and its metabolites A and B, expressed as chloridazon equivalents

If chloridazon is intended to be used on other crops than root crops, further studies are required to set a residue definition for all plant matrices.

B.7.3.2 Residue definition in animal matrices:

The soil metabolite B was identified as main residue in plants and feeding stuff. In metabolism studies on lactating goats and laying hens no further transformation of the substance was observed. Based on this data the residue definition for animal products is proposed as:

Residue definition for monitoring:	Metabolite B, expressed as chloridazon equivalents

The proposed definition of the relevant residue for risk assessment includes metabolite B of chloridazon, as residues of metabolite B in crops were often higher than the residues of parent compound. Since this metabolite does not occur in rats or livestock, separate livestock metabolism studies had been performed, which showed that metabolite B does not undergo metabolic transformation and remains unchanged. The N-glucoside of chloridazon, metabolite A, which is the other relevant plant metabolite, is cleaved in animals and thus considered as being equivalent to the parent compound.

Residue definition for risk assessment:	Sum of chloridazon and metabolite B, expressed as chloridazon equivalents

In the livestock metabolism studies a large amount of dechlorinated chloridazon was found mainly in the muscle and in the liver of lactating goats. The substance was not identified in the rat metabolism study. Nevertheless, the rat metabolism indicates, that dechlorinated chloridazon occurs as an intermediate product. The polarity of the metabolite is higher than the parent substance. Additionally, in animal products no residues of dechlorinated chloridazon above the limit of quantification have to be expected. An inclusion of dechlorinated chloridazon in the residue definition is not considered necessary.

B.7.4 Use pattern

Chloridazon is used as pre-emergence and post-emergence herbicide on beet and onion vegetables. It is further used in fodder beet, which is destined for animal feed. Chloridazon controls a board spectrum of weeds and is used at application rates at 1.3 up to 2.6 kg as/ha depending on the crop and application time. As the product is applied at a very early stage of BBCH 14, there is no waiting period proposed. The application as a herbicide is registered in Northern and Southern European countries.

B.7.5 Identification of critical GAPs

The critical GAPs for the intended uses are similar to each other. The following GAP is representative for all crops.

Table B.7.5-1: Identification of proposed critical GAPs

Crop and/or situation	F or G	Group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)
			Type	conc. of as (g/kg)	method, kind	growth stage	number (range)	kg (as/hL)	water (L/ha)	kg (as/ha)	
Sugar beet, red beet, beta beet, fodder beet, onion, shallot, garlic, flowers and nursery	F	weeds	WG	650	spraying	14	1	1.3	200 – 300	2.6	F

B.7.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.2)

B.7.6.1 Root vegetables

Reports: Keller E., 2000(a)
Summary of residue trials with chloridazon formulations in red beets in 1978
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep.

Keller E., 2000(b)
Summary of residue trials with chloridazon formulations in sugar beets in 1975, 1976, and 1977
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep.

GLP: No, not subject to GLP regulations

Guideline: not specified

Report: Balluff M., 2001
Field Residue Study for the Determination of Residues of the Active Ingredient(s) after the Maximum Number of Applications under Open Field Conditions with BAS 119 33 H in Sugar beet in Northern Europe, 2000
Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH Germany Fed.Rep.

GLP: yes

Guideline: BBA-Guideline part IV, 3-3 (Jan. 1990), Testing of the Behaviour of Residues - General Recommendations for the Design, Preparation and Realisation of Residue Tests European Community Guideline 7029/VI/95-EN - rev. 5, 22/07/97: General recommendations for the design preparation and realisation of residue trials.
IVA Guidelines for Residue Studies, Sections IA and IB, 2nd edition 1992

Reports: Freschi G., 2002(b)
Determination of residues of chloridazon, metabolite A and metabolite B in sugar beet samples (leaves+top, roots and shoots) after an application of chloridazon 65 % WG in two spanish trials, 2001
SIPCAM, Laboratorio Chimico, Salerano sul Lambro, Italy

Freschi G., 2002(c)
Determination of residues of chloridazon, metabolite A and metabolite B in sugar beet samples (leaves+top, roots and shoots) after an application of chloridazon 65 % WG in two Italian trials, 2001
SIPCAM, Laboratorio Chimico, Salerano sul Lambro, Italy

Freschi G., 2002(a)
Determination of residues of chloridazon, metabolite A and metabolite B in sugar beet samples after an application of chloridazon 65 % WG in two Italian trials
SIPCAM, Laboratorio Chimico, Salerano sul Lambro, Italy

Freschi G., 2002(d)
Determination of residues of chloridazon, metabolite A and metabolite B in sugar beet samples after an application of chloridazon 65 % WG in two Spanish trials
SIPCAM, Laboratorio Chimico, Salerano sul Lambro, Italy

GLP: yes

Guideline: Guideline for the generation of data concerning residues as provided in Annex II, part A, Section 6 and Annex III, part A, section 8 of Directive 91/414/EEC concerning the placing of plant protection products on the market. 1607/VI/97 rev. 2, 10.06.99. European Community Guideline 7029/VI/95 – rev.5, 22/07/97: General recommendations for the design preparation and realisation of residue trials.
European Community Guideline 7525/VI/95 – rev. 2, 09/07/98: Comparability, extrapolation, group tolerances and data requirements

Deviations: none

Acceptability: The studies are considered to be acceptable.

Material and Methods:

During the growing seasons 1975 to 1978 and 2000/2001, 30 field trials were conducted in different representative growing areas for root vegetables in Belgium, Germany, Spain, France and Italy (22 in Northern EU, 8 in the South) to determine the residue levels of chloridazon after post emergence application. The trials were performed in sugar beets and red beets.

The herbicidal products were applied once at BBCH growth stage 14 – 16 at an application rate equivalent to 2.6 kg as/ha.

All samples were analysed for chloridazon including metabolite A after hydrolysis and for metabolite B separately. The metabolite B residue was then transformed into parent equivalents, and total residue containing parent and both metabolites A and B was calculated.

Findings:

Table B.7.6-1: Residues of chloridazon and metabolite B in root vegetables

Crop	Application			Residues [1] (mg/kg)					
Country, year (trial no.), Report No.	No.	kg as/ha	kg as/ hL	Matrix	DAT[4]	Chloridazon + Metabolite A	Metabolite B	Metabolite B as parent equiv. [2; 3]	Total [3]
SUGAR BEET									
D – Germany	1	2.6	0.65	whole plant	13	2.15	0.14	0.21	2.36
1975				tops	65	0.29	0.23	0.35	0.64
(11905H75/5A +				tops	101	0.11	0.75	1.14	1.25
11905H75/5AB)				tops	183	< 0.05	0.48	0.73	0.78
				roots	65	< 0.05	< 0.05	< 0.08	< 0.13
#2000/1013266				roots	101	< 0.05	0.08	0.12	0.17
				roots	183	< 0.05	0.07	0.11	0.16
D – Germany	1	2.6	0.65	whole plant	12	0.99	< 0.05	0.08	1.07
1975				tops	64	0.42	0.12	0.18	0.60
(11905H75/6+				tops	99	0.13	0.29	0.44	0.57
11905H75/6AB)				tops	147	< 0.05	0.38	0.58	0.63
				roots	64	0.06	< 0.05	0.08	0.14
#2000/1013266				roots	99	< 0.05	0.08	0.12	0.17
				roots	147	< 0.05	0.05	0.08	0.13
D – Germany	1	2.6	0.65	whole plant	22	0.34	< 0.05	0.08	0.42
1975				tops	63	0.72	0.05	0.08	0.80
(11905H75/7+				tops	93	0.30	0.32	0.49	0.79
11905H75/7AB)				tops	138	< 0.05	0.30	0.46	0.51
				roots	63	0.09	< 0.05	0.08	0.17
#2000/1013266				roots	93	< 0.05	0.05	0.08	0.13
				roots	138	< 0.05	0.05	0.08	0.13
D – Germany	1	2.6	0.65	whole plant	16	0.28	0.10	0.15	0.43
1975				tops	28	0.10	0.12	0.18	0.28
(11905H75/8+				tops	55	< 0.05	0.82	1.25	1.30
11905H75/8AB)				tops	98	< 0.05	0.22	0.33	0.38
				roots	28	< 0.05	0.07	0.11	0.16
#2000/1013266				roots	55	< 0.05	0.07	0.11	0.16
				roots	98	< 0.05	0.06	0.09	0.14
D – Germany	1	2.58	0.65	tops	12	1.63	< 0.05	0.08	1.71
1976				tops	41	0.23	0.05	0.08	0.31
(11916H76/3A+				tops	74	< 0.05	0.06	0.09	0.14
11916H76/3AB)				tops	104	0.06	0.22	0.33	0.39
				roots	74	0.05	< 0.05	0.08	0.13
#2000/1013266				roots	104	< 0.05	0.05	0.08	0.13

Crop	Application			Residues [1] (mg/kg)					
Country, year (trial no.), Report No.	No.	kg as/ha	kg as/ hL	Matrix	DAT[4]	Chloridazon + Metabolite A	Metabolite B	Metabolite B as parent equiv. [2; 3]	Total [3]
D – Germany	1	2.58	0.78	tops	14	0.15	0.32	0.49	0.64
1976				tops	62	0.11	0.28	0.43	0.54
(11916H76/7A+				tops	95	0.23	0.51	0.78	1.01
11916H76/3A				tops	144	< 0.05	0.36	0.55	0.60
				roots	62	0.06	0.52	0.79	0.85
#2000/1013266				roots	95	0.05	0.06	0.09	0.14
				roots	144	< 0.05	0.10	0.15	0.20
D – Germany	1	2.58	0.78	tops	7	1.50	0.14	0.21	1.71
1976				tops	23	7.20	0.17	0.26	7.46
(11916H76/11)				tops	55	0.29	0.05	0.08	0.37
				tops	104	0.08	< 0.05	0.08	0.16
#20001013266				roots	55	0.20	0.20	0.30	0.50
				roots	104	< 0.05	0.05	< 0.08	< 0.13
D – Germany	1	2.58	0.64	tops	9	6.08	< 0.05	0.08	6.16
1976				tops	64	0.44	< 0.05	0.08	0.52
(11916H76/15)				tops	92	0.17	< 0.05	0.08	0.25
				tops	167	< 0.05	0.31	0.47	0.52
#2000/1013266				roots	64	< 0.05	< 0.05	< 0.08	< 0.13
				roots	92	0.05	< 0.05	0.08	0.13
				roots	167	< 0.05	< 0.05	< 0.08	< 0.13
D – Germany	1	2.58	0.64	tops	12	2.17	0.13	0.20	2.37
1977				tops	64	0.35	0.27	0.41	0.76
(11916H77/2)				tops	119	0.05	0.52	0.79	0.84
				tops	156	< 0.05	0.30	0.46	0.51
#2000/1013266				roots	64	0.07	< 0.05	0.08	0.15
				roots	119	< 0.05	0.10	0.15	0.20
				roots	156	< 0.05	< 0.05	< 0.08	< 0.13
D – Germany	1	2.58	0.64	tops	0	71.70	0.09	0.14	71.84
1977				tops	28	0.26	0.63	0.96	1.22
(11916H77/6)				tops	83	< 0.05	0.24	0.37	0.42
				tops	111	< 0.05	0.48	0.73	0.78
#2000/1013266				roots	83	< 0.05	0.06	0.09	0.14
				roots	111	0.06	0.08	0.12	0.18
D – Germany	1	2.58	0.78	tops	35	1.65	< 0.05	0.08	1.73
1977				tops	69	0.11	< 0.05	0.08	0.19
(11916H77/10)				tops	125	< 0.05	0.76	1.16	1.21
				tops	150	< 0.05	0.42	0.64	0.69
#2000/1013266				roots	69	< 0.05	< 0.05	< 0.08	< 0.13
				roots	125	< 0.05	0.06	0.09	0.14
				roots	150	< 0.05	< 0.05	< 0.08	< 0.13
D – Germany	1	2.58	0.64	tops	14	0.60	0.08	0.12	0.72
1977				tops	39	0.16	0.33	0.50	0.66
(11916H77/14)				tops	95	< 0.05	0.56	0.85	0.90
				tops	132	< 0.05	0.23	0.35	0.40
#2000/1013266				roots	39	< 0.05	0.15	0.23	0.28
				roots	95	< 0.05	0.08	0.12	0.17
				roots	132	< 0.05	< 0.05	< 0.08	< 0.13
B - Belgium	1	2.6	0.87	shoots	0	2.57	0.259	0.39	2.96
2000				shoots	24	0.087	0.115	0.17	0.26
(B00W001R)				shoots	52	< 0.05	0.100	0.15	0.20
				tops	125	< 0.05	0.128	0.19	0.24
#2001/1009078				roots	125	< 0.05	< 0.05	< 0.08	< 0.13

Crop	Application			Residues [1] (mg/kg)					
Country, year (trial no.), Report No.	No.	kg as/ha	kg as/ hL	Matrix	DAT[4]	Chloridazon + Metabolite A	Metabolite B	Metabolite B as parent equiv. [2; 3]	Total [3]
B - Belgium	1	2.6	0.87	shoots	0	8.11	0.091	0.14	8.25
2000				shoots	21	0.276	0.087	0.13	0.41
(NL00W001R)				shoots	49	0.051	0.087	0.13	0.18
				tops	122	< 0.05	0.128	0.19	0.24
#2001/1009078				roots	122	< 0.05	< 0.05	0.08	< 0.13
F - France	1	2.6	0.87	shoots	0	13.51	0.069	0.10	13.61
2000				shoots	27	0.063	0.090	0.14	0.20
(F00W037R)				shoots	54	< 0.05	0.091	0.14	0.19
				tops	124	< 0.05	0.393	0.60	0.65
#2001/1009078				roots	124	< 0.05	< 0.05	< 0.08	< 0.13
F - France	1	2.6	0.87	shoots	0	7.37	0.052	0.08	7.45
2000				shoots	23	0.654	< 0.05	0.08	0.73
(F00W038R)				shoots	63	< 0.05	0.077	0.12	0.17
				tops	142	< 0.05	0.177	0.27	0.32
#2001/1009078				roots	142	< 0.05	< 0.05	< 0.08	< 0.13
E – Spain	1	2.6	0.87	leaves + top	155	< 0.05	0.072	0.11	0.16
2000				roots	155	< 0.05	0.05	0.08	0.13
(CH1/S/03SB)									
#2002/1011937									
E – Spain	1	2.6	0.87	leaves + top	155	< 0.05	< 0.05	< 0.08	< 0.13
2000									
(CH1/S/04SB)				roots	155	< 0.05	0.05	0.08	0.13
#2002/1011937									
E – Spain	1	2.6	0.87	leaves + top	131	< 0.05	0.105	0.16	0.21
2001				roots	131	< 0.05	< 0.05	0.08	< 0.13
(CZ1/SB/S-01)									
E – Spain	1	2.6	0.87	leaves + top	132	< 0.05	< 0.05	0.08	< 0.13
2001									
(CZ1/SB/S-02)				roots	132	< 0.05	< 0.05	0.08	< 0.13
#2002/1011935									
I – Italy	1	2.6	0.87	leaves + top	118	< 0.05	0.52	0.79	0.84
2000				roots	118	< 0.05	0.05	0.08	0.13
(CH1/I/01SB)									
I – Italy	1	2.6	0.87	leaves + top	106	< 0.05	0.39	0.59	0.64
2000									
(CH1/I/02SB)				roots	106	< 0.05	0.05	0.08	0.13
#2002/1011934									
I – Italy	1	2.6	0.87	leaves + top	91	< 0.05	0.17	0.26	0.31
2001									
(CH2/I/01SB)				roots	91	< 0.05	< 0.05	< 0.08	< 0.13
#2002/1011934									
I – Italy	1	2.6	0.87	leaves + top	98	< 0.05	0.10	0.16	0.21
2001									
(CH2/I/02SB)				roots	98	< 0.05	< 0.05	< 0.08	< 0.13
2002/1011936									
RED BEET									
D – Germany	1	2.6	0.65	tops	65	0.28	0.69	1.05	1.33
1978				roots	65	< 0.05	0.11	0.17	0.22
(11985H78/1A)				tops	79	0.18	0.57	0.87	1.05
				roots	79	< 0.05	0.07	0.11	0.16
#2000/1013265				tops	86	0.24	0.66	1.00	1.24
				roots	86	< 0.05	0.08	0.12	0.17
				tops	93	0.33	0.75	1.14	1.47
				roots	93	< 0.05	< 0.05	< 0.08	< 0.13

Crop	Application			Residues [1] (mg/kg)					
Country, year (trial no.), Report No.	No.	kg as/ha	kg as/ hL	Matrix	DAT[4]	Chloridazon + Metabolite A	Metabolite B	Metabolite B as parent equiv. [2; 3]	Total [3]
D – Germany	1	2.6	0.65	tops	65	0.37	0.36	0.55	0.92
1978				roots	65	< 0.05	< 0.05	< 0.08	< 0.13
(11905H78/2A)				tops	79	0.22	0.23	0.35	0.57
				roots	79	< 0.05	0.05	0.08	0.13
#2000/1013265				tops	86	0.19	0.72	1.10	1.29
				roots	86	< 0.05	0.12	0.18	0.23
				tops	93	0.35	0.67	1.02	1.37
				roots	93	< 0.05	0.07	0.11	0.16
D – Germany	1	2.6	0.65	tops	65	0.32	0.55	0.84	1.16
1978				roots	65	< 0.05	< 0.05	< 0.08	< 0.13
(11905H78/3A)				tops	79	0.31	0.47	0.71	1.02
				roots	79	< 0.05	< 0.05	< 0.08	< 0.13
#2000/1013265				tops	86	0.20	0.76	1.16	1.36
				roots	86	< 0.05	0.05	0.08	0.13
				tops	93	0.18	0.38	0.58	0.76
				roots	93	< 0.05	0.06	0.09	0.14
D – Germany	1	2.58	0.64	tops	65	0.42	0.47	0.71	1.13
1978				roots	65	< 0.05	0.07	0.11	0.16
(11905H78/13A)				tops	79	0.42	0.62	0.94	1.36
				roots	79	< 0.05	0.07	0.11	0.16
#2000/1013265				tops	86	0.23	0.43	0.65	0.88
				roots	86	< 0.05	0.10	0.15	0.20
				tops	93	0.16	0.46	0.70	0.86
				roots	93	< 0.05	0.09	0.14	0.19
D – Germany	1	2.58	0.64	tops	65	0.33	0.06	0.09	0.42
1978				roots	65	< 0.05	0.05	0.08	0.13
(11905H78/14A)				tops	79	0.14	0.26	0.40	0.54
				roots	79	< 0.05	< 0.05	< 0.08	< 0.13
#2000/1013265				tops	86	0.47	0.85	1.29	1.76
				roots	86	< 0.05	0.08	0.12	0.17
				tops	93	0.27	0.64	0.97	1.24
				roots	93	< 0.05	0.07	0.11	0.16
D – Germany	1	2.58	0.64	tops	65	0.27	0.48	0.73	1.00
1978				roots	65	< 0.05	0.05	0.08	0.13
(11905H78/15A)				tops	79	0.23	0.33	0.50	0.73
				roots	79	0.05	0.05	0.08	0.13
#2000/1013265				tops	86	0.38	0.93	1.41	1.79
				roots	86	< 0.05	0.08	0.12	0.17
				tops	93	0.16	0.30	0.46	0.62
				roots	93	< 0.05	0.07	0.11	0.16

[1]) Residues are not corrected for recoveries
[2]) The factor for calculating metabolite B to parent chloridazon is 1.521
[3]) For calculation purposes, "< 0.05" is set "0.05"
[4]) DAT = Days After Treatment

Residues in sugar beet roots after 98 to 183 days:
<0.13(13), 0.13(7), 0.14, 0.16, 0.18, 0.20 mg/kg chloridazon/metabolite A + metabolite B, calculated as chloridazon

STMR: 0.13 mg/kg HR: 0.20 mg/kg

Residues in sugar beet tops/leaves after 98 to 183 days:
<0.13, <0.13, 0.16, 0.16; 0.21, 0.21, 0.24(2),
0.31, 0.32, 0.37, 0.38, 0.39, 0.40, 0.51, 0.51,
0.52, 0.60, 0.63, 0.64, 0.65, 0.69, 0.78, 0.78,
0.84 mg/kg — chloridazon/metabolite A + metabolite B, calculated as chloridazon

STMR: 0.39 mg/kg HR: 0.84 mg/kg

Residues in red beet roots after 93 days:
<0.13, 0.14, 0.16(3), 0.19 mg/kg — chloridazon/metabolite A + metabolite B, calculated as chloridazon

STMR: 0.16 mg/kg HR: 0.19 mg/kg

Residues in red beet tops after 93 days:
0.62, 0.76, 0.86, 1.2, 1.4, 1.5 mg/kg — chloridazon/metabolite A + metabolite B, calculated as chloridazon

STMR: 1.03 mg/kg HR: 1.5 mg/kg

B.7.6.2 Onion

Report: Raunft E., 2003
Study on the residue behaviour of chloridazon in onions after treatment with BAS 119 33 H under field conditions in Germany and Great Britain, 2000
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep.

GLP: yes

Guideline: UK Guidance on Crop Residue Data Requirements - Data requirements for Approval under the Control of Pesticides Regulations 1986, Appendix 6, PSD, October 1992
BBA-Guideline part IV, 3-3 (Jan. 1990), Testing of the Behaviour of Residues - General Recommendations for the Design, Preparation and Realisation of Residue Tests
IVA Guidelines for Residue Studies, Sections IA and IB, 2nd edition 1992
Guidelines on Producing Pesticide Residue Data from Supervised Trials, FAO Rome, 1990

Report: Jones S., 2003(a)
Study on the residue behaviour of BAS 119 H in onion after application of BAS 119 33 H under field conditions in Belgium, Denmark, Sweden 2001
BASF plc, BASF Agro Research, Gosport, Hampshire United Kingdom

Jones S., 2003(b)
Final report amendment: Study on the residue behaviour of BAS 119 H in onion after application of BAS 119 33 H under field conditions in Belgium, Denmark, Sweden 2001
BASF plc, BASF Agro Research, Gosport, Hampshire United Kingdom

GLP: yes

Guideline: UK Guidance on Crop Residue Data Requirements – Data requirements for Approval under the Control of Pesticides Regulations 1986, Appendix 6, PSD, October 1992
Guideline for the generation of data concerning residues as provided in Annex II, part A, Section 6 and Annex III, part A, section 8 of Directive 91/414/EEC concerning the placing of plant protection products on the market. 1607/VI/97 rev. 2, 10.06.99.
European Community Guideline 7029/VI/95 – rev.5, 22/07/97: General recommendations for the design preparation and realisation of residue trials.
European Community Guideline 7525/VI/95 – rev. 2, 09/07/98: Comparability, extrapolation, group tolerances and data requirements

Deviations: none

Acceptability: The studies are considered to be acceptable.

Material and Methods:
During the 2000 and 2001 growing seasons, 8 field trials were conducted in different representative onion growing areas in Belgium, Germany, Denmark, Great Britain and Sweden to determine the residue levels of chloridazon.
The test item chloridazon was applied at GS 10 (BBCH code for onions) with an application rate of 4.0 kg/ha in a spray solution of 300 L/ha.
The trials in Belgium, Denmark and Sweden were analysed with two methods, one of them analysed chloridazon separately and the other one the sum of chloridazon and metabolite A.

Findings:

Table B.7.6-2: Residues of chloridazon, metabolite A and metabolite B in onion

Crop	Application			Residues (mg/kg)					
Country, year (trial no.), Report No.	No.	kg as/ha	kg as/ hL	Matrix	DAT	Chloridazon + Metabolite A	Metabolite B	Metabolite B as parent equiv.	Total
ONION									
D – Germany 2000	1	2.6	0.87	pl. w/o root	49	< 0.05	< 0.05	< 0.08	< 0.13
(ACK/08/00)				bulb	108	< 0.05	< 0.05	< 0.08	< 0.13
#2003/1000943									
D – Germany 2000	1	2.6	0.87	pl. w/o root	29	0.28	0.06	0.09	0.37
(DU4/09/00)				bulb	78	< 0.05	< 0.05	< 0.08	< 0.13
#2003/1000943									
GB – Great Britain 2000	1	2.6	0.87	pl. w/o root	91	< 0.05	< 0.05	< 0.08	< 0.13
(OAT/18/00)				bulb	122	< 0.05	< 0.05	< 0.08	< 0.13
#2003/1000943									
GB – Great Britain 2000	1	2.6	0.87	pl. w/o root	77	< 0.05	0.08	0.12	0.17
(OAT/19/00)				bulb	112	< 0.05	< 0.05	< 0.08	< 0.13
#2003/1000943									

Table B.7.6-3: Residues of chloridazon and metabolite B in onion

Crop	Application			Residues (mg/kg)						
Country, year (trial no.), Report No.	No.	kg as/ha	kg as/ hL	Matrix	DAT	Chloridazon	Chloridazon + Metabolite A	Metabolite B	Metabolite B as parent equiv.	Total
ONION										
B – Belgium 2001	1	2.6	0.87	pl. w/o root	72	<0.05	< 0.05	0.10	0.15	0.20
(AGR/01/01)				bulb	108	<0.05	< 0.05	< 0.05	< 0.08	< 0.13
#2003/1000944										
B – Belgium 2001	1	2.6	0.87	pl. w/o root	72	<0.05	0.06	0.46	0.70	0.76
(AGR/02/01)				bulb	108	<0.05	< 0.05	0.06	0.09	0.14
#2003/1000944										
DK – Denmark 2001	1	2.6	0.87	pl. w/o root	42	0.21	0.39	< 0.05	< 0.08	0.47
(ALB/01/01)				bulb	119	<0.05	< 0.05	< 0.05	< 0.08	< 0.13
#2003/1000944										
S – Sweden 2001	1	2.6	0.87	bulb	146	<0.05	< 0.05	< 0.05	< 0.08	< 0.13
(HUS/01/01)										
#2003/1000944										

Residues in onion bulbs after 78 to 122 days:
<0.13(3), 0.14 mg/kg chloridazon/metabolite A + metabolite B, calculated as chloridazon
STMR: 0.13 mg/kg HR: 0.14 mg/kg

Residues in onion bulbs after 108 to 146 days:
<0.13(4) mg/kg chloridazon + metabolite B, calculated as chloridazon
STMR: 0.13 mg/kg HR: 0.13 mg/kg

B.7.6.3 Storage stability

Report: Riley M., Movassaghi S., 1996
Freezer storage stability of chloridazon (pyrazon) and metabolites in sugar beet roots, tops, dried pulp, refined sugar, and molasses.
BASF Corporation Agricultural Products Center, Research Triangle Park, NC 27709, USA

GLP: yes

Guideline: EPA Guidelines, Subdivision O, 171-4(e)

Deviations: none

Acceptability: The study is considered to be acceptable.

Material and Methods:
The deep freeze stability of chloridazon and its metabolites A and B in various sugar beet matrices was investigated over a period of two years. Untreated samples were fortified with 1.0 mg/kg each of the three analytes. The samples were stored frozen (< -5 °C) and analysed in comparison with freshly fortified samples after 3-4, 6-7, 12-14 and 24-28 months.

Findings:

Table B.7.6-4: Amount of chloridazon found in sugar beet matrices (fortification level 1.0 mg/kg)

Matrix	Time (months)	Chloridazon found (mg/kg)			
		in stored samples		in freshly fortified samples	
Sugar beet root	0	-	-	1.040	1.170
	3	0.941	0.805	1.100	1.030
	6	0.960	0.800	1.040	1.100
	12	0.679	0.681	0.912	0.806
	27	0.680	0.674	0.799	0.801
		0.723	0.702	0.945	0.880
Sugar beet top	0	-	-	0.925	0.964
	4	0.802	0.791	0.739	0.722
	6	0.705	0.848	0.885	0.823
	13	0.795	0.735	0.786	0.659
	27	0.604	0.695	0.730	0.733
Refined sugar	0	-	-	0.884	1.010
	3	0.923	0.904	0.939	1.050
	6	0.821	0.789	0.898	1.160
	12	0.787	0.855	0.786	0.910
	27	0.871	0.956	0.833	0.883
Molasses	0	-	-	1.010	0.990
	3	0.938		0.843	0.726
	7	0.843	0.845	0.899	0.865
	14	0.724	0.809	0.906	0.941
	27	1.122	1.055	0.840	0.943
Dried pulp	0		-	0.826	0.900
	3	0.773	0.778	0.759	0.814
	6	0.777	0.796	0.797	0.851
	12	0.802	0.867	0.859	0.770
	24	0.660	0.734	0.797	0.879

Table B.7.6-5: Amount of metabolite B found in sugar beet matrices (fortification level 1.0 mg/kg)

Matrix	Time (months)	Metabolite B found (mg/kg)			
		in stored samples		in freshly fortified samples	
Sugar beet root	0	-	-	0.845	0.803
	3	0.749	0.746	1.000	0.931
	6	0.643	0.682	0.796	0.771
	12	0.756	0.724	0.861	0.712
	27	0.793	0.783	0.835	0.810
Sugar beet top	0	-	-	0.863	0.971
	4	0.850	1.210	0.821	0.789
	7	0.832	0.849	0.801	0.833
	12	0.707	0.700	0.728	
	28	0.634	0.609	0.725	0.751
Refined sugar	0	-	-	1.140	1.130
	3	0.702	0.826	0.762	0.803
	7	0.787	0.762	0.810	0.679
	12	0.735	0.736		0.801
	27	0.662	0.782	0.795	0.836
Molasses	0	-	-	0.793	0.801
	3	0.870	1.033	0.951	0.829
	7	0.925	0.875	1.021	1.018
	14	0.688	0.876	0.898	0.820
	27	1.139	1.296	0.873	0.913
Dried pulp	0	-	-	0.749	0.737
	3	0.764	0.804	0.778	0.796
	6	0.780	0.789	0.777	0.844
	12	0.677	0.790	0.716	0.730
	24	0.815	0.852	0.810	0.838

Table B.7.6-6: Amount of metabolite A found in sugar beet matrices (fortification level 1.0 mg/kg)

Matrix	Time (months)	Metabolite A found (mg/kg)			
		in stored samples		in freshly fortified samples	
Sugar beet root	0	-	-	0.857	0.717
	3	0.826	0.736	0.872	0.792
	6	0.923	0.802	0.788	0.846
	12	0.759	0.691	0.743	0.887
	27	0.855	0.907	0.711	0.758
Sugar beet top	0	-	-	0.773	0.766
	3	0.793	0.709	0.883	0.691
	6	0.911	0.766	0.859	0.714
	12	0.832	0.832	0.921	0.943
	28	0.630	0.647	0.695	0.692
		0.948	0.998	0.799	0.843
Refined sugar	0	-	-	0.823	0.835
	3	1.040	0.815	0.993	0.903
	6	0.917	0.721	0.793	0.928
	12	0.775	0.730	0.779	0.715
	27	0.751	0.742	0.742	0.725
Molasses	0	-	-	0.721	0.805
	3	0.862	0.941	0.763	0.836
	7	1.016	0.900	0.777	0.855
	14	0.851	0.924	0.787	0.735
	27	0.687	0.735	0.623	0.751
Dried pulp	0	-	-	0.782	0.818
	3	0.730	0.706	0.843	0.846
	6	0.621	0.702	0.651	0.746
		0.730	0.785	0.766	0.837
	12	0.903	0.742	0.830	0.820
	24	1.33	1.18	1.04	1.13

Conclusion:
The results indicate that chloridazon and the main metabolites A and B are stable over a storage period of 24 months.

B.7.7 Effects of industrial processing and/or household preparation (Annex IIA 6.5; Annex IIIA 8.4)

B.7.7.1 Effects on the nature of residue

Report: von Goetz N., 2001
Hydrolysis of BAS 119 H and metabolite B of chloridazon at 90 °C, 100 °C, and 120 °C
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep.

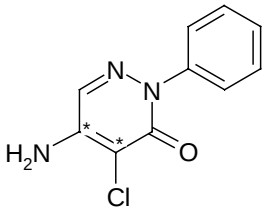
GLP: yes

Guideline: Appendix 1 to § 19a, Section 1, Chemikaliengesetz of July 1994 (Official Bulletin/Federal Republic of Germany, I 1994, P.1703 Council Directive 91/414/EEC

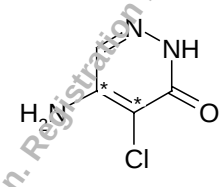
Deviations: none

Acceptability: The study is considered to be acceptable.

The effects on the nature of residues in crops were investigated using ^{14}C-labels of the test substance chloridazon and metabolite B. The molecular structures and the positions of the labels are shown below:



Chloridazon



Metabolite B

* denotes position of the ^{14}C-label

Material and Methods:
To estimate the degradation behaviour of the test substance during industrial processing or household preparation, different processes (pasteurisation, baking, brewing, boiling and sterilisation) have to be simulated. The amount and nature of products formed during the different processes have to be determined.
Chloridazon is intended to be used in sugar beet and red beet. The processing of both crops include boiling and for canning even sterilisation. Hence these processes were simulated.
The test substance was dissolved in aqueous buffer solutions of different pH-values. To avoid an influence of light, the glassware was wrapped. The test solutions were pasteurised for 20 min at 90 °C, boiled for 60 min at 100 °C in a four-neck round-bottom flask under reflux and a box with 14 sample vessels was sterilised at about 120 °C in an autoclave for 20 min.
All samples of the test solutions were analysed directly without work-up. If necessary, samples were stored in a freezer before analysis. All samples were measured for radioactivity (LSC) and were analysed by HPLC to determine a metabolite pattern.

Findings:
The purity was ≥98 % for chloridazon and 100 % for metabolite B. Hence, no purification step had to be performed.
As shown in Table B.7.7-1 the values for the recovery range between about 96 and 104 %.

Table B.7.7-1: Quantification of degradation products after simulation of pasteurisation, baking, boiling and sterilisation

Test description	Status	Total Recovery in %	
		Chloridazon	Metabolite B
pH 4, 90 °C, 20 min	before test	100.0	100.0
	after test	95.7	95.6
pH 5, 100 °C, 60 min	before test	100.0	100.0
	after test	103.9	100.7
pH 6, 120 °C, 20 min	before test	100.0	100.0
	after test	103.7	96.1

Conclusion:
The results of this study indicate that chloridazon and the metabolite B are stable under simulated processing conditions.

B.7.7.2 Effects on the residue level

Report: Schulz H., 2001
Determination of the residues of chloridazon in sugar beet and processed products following treatment with BAS 119 33 H under field conditions in Germany, 2000
Institut Fresenius, Chemische und Biologische Laboratorien GmbH, Taunusstein, Germany Fed. Rep.

GLP: yes

Guideline: BBA Guideline Part IV, 3-3, Testing of residue behaviour - General information on design, preparation and realisation of residue tests -,
BBA Guideline Part IV, 3-4, "Testing the residue behaviour - Residue tests on processed plant products (processing guideline)
IVA Guideline for Residue Studies, Sections IA and IB, 2nd edition 1992,
European Community Guideline 7035/VI/95 – rev.5, 22/07/97: Processing studies
EC Guidance Document on Residue Analytical Methods (SANCO/825/00, rev.6)
EC guideline 96/46/EC dated July 16, 1996.

Deviations: none

Acceptability: The study is considered to be not acceptable.

Material and Methods:
During the season 2000 four field trials were carried out at four locations in Germany. The plots were treated once with 5.2 kg/ha of chloridazon. The applications were carried at BBCH growth stage 14. For the residue analysis samples of whole plants of sugar beets were taken manually between 13 and 17 days after treatment (DAT) and at harvest time (151 and 157 DAT). These samples were divided into leaves with tops and roots.

The processing of the sugar beets was carried out in a pilot plant of Technische Universität Berlin, Germany, simulating commercial practice. The sugar beets were processed to the following products:
Dirt and dirty water, cossettes (RAC), pressed pulp, press water, raw juice, thin juice, mud, thick juice, raw sugar, molasses, affination syrup, white sugar

Findings:
At 13 – 17 DAT, the residues of total chloridazon ranged between 0.916 and 3.936 mg/kg. At harvest (151 to 157 DAT), the residues considerably decreased. In leaves with tops, 0.516 to 0.668 mg/kg were found. The levels in root were below or close to the limit of quantification of the method applied; they ranged from <0.126 to 0.141 mg/kg.
Regarding the processed fractions, the total chloridazon residues in the cossettes were <0.126 mg/kg, which were the starting concentrations in the RAC materials used for processing. In dirt and dirty water (not a processed product of the cossettes, since these samples were taken after washing of the field samples), pressed pulp, press water, raw juice, thin juice, mud, raw sugar, affination syrup and white sugar, no residues of total chloridazon above the limit of quantification of 0.126 mg/kg were obtained. In thick juice, the residues ranged between <0.126 and 0.355 mg/kg, resulting in transfer factors between 1 and 2.8. In molasses, the residues ranged from 0.400 to 1.136 mg/kg, resulting in transfer factors between 3.2 and 9.0.
The residues of total chloridazon in matrices of treated sugar beets and processed fractions as well as the corresponding transfer factors were as follows, whereby the total residue concentrations of the cossettes were set = 1:

Table B.7.7-2: Residue concentrations and transfer factors

Sample	DAT	Total chloridazon	
		mg/kg	Transfer factor
Whole plant	13 - 17	0.916 - 3.936	---
Leaves with tops	151 - 157	0.516 - 0.668	---
Roots	151 - 157	< 0.126 - 0.141	---
Cossettes (RAC)	151 - 157	< 0.126	1
Dirt and dirty water	---	< 0.126	---
Pressed pulp	---	< 0.126	1
Press water	---	< 0.126	1
Raw juice	---	< 0.126	1
Thin juice	---	< 0.126	1
Mud	---	< 0.126	1
Thick juice	---	< 0.126 - 0.355	1, 2.1, 2.2, 2.8
Raw sugar	---	< 0.126	1
Molasses	---	0.400 - 1.136	3.2, 3.3, 6.1, 9.0
Affination syrup	---	< 0.126	1
White sugar	---	< 0.126	1

Remarks:

a) For calculation purposes, < 0.05 mg/kg of Chloridazon was set as 0.05 mg/kg.
b) For calculation purposes, < 0.05 mg/kg of metabolite B was set as 0.05 mg/kg.
c) Total chloridazon means the sum of chloridazon, metabolite A (if any) and metabolite B, whereby the conversion factor from the residue concentration of metabolite B to chloridazon is 1.5.

Discussion:
This study to measure the effect on residue levels during processing of sugar beets does not give reliable transfer factors for the different industrial procedures. The cossettes as raw agricultural commodities have a residue level below the LOQ of <0.126 mg/kg. Therefore, a

calculation of transfer factors is not possible. The transfer factors calculated in the study can only be taken as a tendential value for processing.

B.7.8 Livestock feeding studies (Annex IIA 6.4; Annex IIIA 8.3)

B.7.8.1 Ruminants

Report: Zehr R.D., Riley M., 1996
A meat and milk magnitude of the residue study with BAS 119 H (chloridazon) in lactating dairy cows
[redacted]

GLP: yes

Guideline: EPA 171-4(j)

Deviations: none

Acceptability: The study is considered to be acceptable.

Material and Methods:
Fourteen lactating Holstein dairy cows aged between 4 and 7 years and in the weight range of 580 kg to 800 kg before treatment were used in the study. The test item was administered twice daily on 29 consecutive days at three dose levels. It was administered orally in capsules by means of a balling gun. Animals of the control group received cellulose-containing capsules.

Feeding and husbandry
The animals were housed individually in outdoor sand-floor pens under usual husbandry conditions. They were allowed *ad libitum* access to a commercial concentrate (total mixed ration) and fresh potable tap water daily via individual feeders and automatic waterers. Individual daily feed consumption was recorded for each animal from study day -8 (approximately one week before the first dose) until the end of the study.

Derivation of selected dose levels
The feeding levels were estimated based on the available residue data for sugar beets and information from processing studies. The calculation according to the US EPA table is shown in Table B.7.8-1.

Table B.7.8-1: Calculation of the feed burden for cattle (according to the US EPA table)

Feed Item	% dry matter	% in diet	Residue level [mg/kg]	Dietary burden [mg/kg in feed]
Dairy Cattle				
Sugar beet leaves	23	10	2.000	0.870
Molasses	[6x] [1)]	10	0.500 [2)]	0.300
Dry Pulp	88	20	0.2	0.045
Total diet [mg/kg]				1.215
Beef Cattle				
Sugar beet leaves	23	20	2.000	1.739
Molasses	[6x] [1)]	10	0.500 [2)]	0.300
Dry Pulp	88	20	0.2	0.045
Total diet [mg/kg]				2.085

[1]) Processing factor
[2]) Residue level in RAC

Dose preparation

The test item was weighed into gelatine capsules, which were then sealed and labelled for each animal with the animal number and a capsule number for identification. Capsules were prepared weekly and stored at <10 °C prior to use. Individual doses were calculated from each animal's average daily feed intake (dry weight basis) from the previous week, multiplying the feed intake by the dose rate and correcting for purity. Placebo capsules filled with cellulose were used for the control group.

Dose administration

Animals were dosed once daily. The achieved daily intake is listed in Table B.7.8-2. The actual doses were calculated on a mg/kg b.w., a mg/kg dry feed and a mg/animal/day basis, taking into account the individual body weights in the beginning and at the end of the dosing period, the actual average feed intake (determined daily), and the actual amount of the test item given to the animals (determined daily). The dosing period was of four weeks duration (29 days). Three cows in each test group were sacrificed within six hours after the final dose administration (zero withdrawal). Two cows of dose group IV were maintained on basal diet and were sacrificed after two and five days withdrawal.

Table B.7.8-2: Calculation of the actual dose levels

Cow (BASF Number)	Nominal dose [mg/kg feed]	Actual dose		
		[mg/animal/day] [1]	[mg/kg bw] [2]	[mg/kg feed] [3]
823 (1)	0	0	0	0
827 (2)	0	0	0	0
838 (3)	0	0	0	0
Average Group I	0	0	0	0
825 (4)	2.5	49.6	0.09	2.68
828 (5)	2.5	45.0	0.07	2.73
836 (6)	2.5	39.0	0.05	2.90
Average Group II	2.5	44.5	0.07	2.77
832 (7)	7.5	147.7	0.24	8.30
837 (8)	7.5	113.5	0.16	7.00
835 (9)	7.5	148.0	0.21	7.88
Average Group III	7.5	136.4	0.20	7.73
830 (10)	25	352.1	0.48	26.55
831 (11)	25	569.4	0.93	26.35
840 (12)	25	520.1	0.73	27.58
833 (13)*	25	492.9	0.63	25.15
829 (14)*	25	617.6	0.77	28.78
Average Group IV	25	510.4	0.71	26.88

[1]) based on averaged individual daily doses
[2]) based on averaged individual body weights of days –1 and 30
[3]) based on averaged individual daily feed intake
*) animals from withdrawal group, calculation for dosing period only

Milking and milk sampling

All cows were machine milked twice daily into individual bucket units and the milk yield (kg) was recorded. For each animal, milk collected in the afternoon was refrigerated over night, sub-sampled, and combined with a sub-sample of the milk collected in the morning. The afternoon and morning sub-samples were proportionally equal, relative to the total milk collected at each time point. For example, 700 g of the total 10,668 g of the afternoon milk were combined with 997 g of the total 15,192 g of the morning milk. The afternoon and morning milk samples were collected daily and analyses were carried out on samples of study days –1, 1, 4, 7, 10, 13, 16, 19, 22, 25 and 28 of all treatment groups. Additional intervals were analysed at study days 2, 3 and 5 for dose group I (control), and on days 29, 31 and 33 for animals #13 and #14 of Group IV in order to monitor residue decline. All animals maintained normal milk production during the study except for Cow #837 (8), which became sick. Her milk production dropped on study day 8, but increased after day 12 and stabilised on day 20. No treatment-related group differences were observed.
Two milk samples of animals of Group IV from study days 19 and 29 (chosen for their higher residues) were separated into cream and skim milk by centrifugation.

Terminal procedures

Animals of Group I were sacrificed prior to the other groups to avoid cross contamination. The animals were terminated by first stunning via a captive bolt pistol followed immediately by exsanguination. At necropsy, tissues were dissected and grossly examined. Following the examination, samples of muscle (composite of thigh and loin, 1 kg total), liver (composite of distal portion of each lobe, 2 kg total), kidney (both) and fat (composite of mesenteric and peripheral, 1 kg total) were collected. The samples were chopped, weighed, placed in labelled storage bags and frozen immediately until transferred for analysis.

Findings:

Bodyweight

Animals of Group I (control) and Group IV (10 x dose level) showed a slight weight gain during the course of the experimental period while the average bodyweights of animals in Groups II and III decreased slightly. Bodyweight changes were considered to be within normal limits and no treatment-related group differences were observed.

Residue analysis

Analysis of the samples determines the residues of chloridazon and 4-hydroxy chloridazon.
Principle of the method: Tissue samples are extracted with methanol. Upon addition of water, the organic solvent is evaporated and the aqueous residue partitioned with hexane followed by ethyl acetate. The ethyl acetate extract is passed through a silica gel column and separated into 4-hydroxy chloridazon and chloridazon containing fractions, the latter one being further purified on a C18 SPE cartridge. Chloridazon is determined by gas chromatography and the metabolite 4-hydroxy chloridazon by HPLC.
Milk samples (whole milk, skim milk and cream) are treated with acetone/acetonitrile. After addition of water the organic extract is concentrated to the aqueous phase, which is partitioned with hexane and then refluxed with hydrochloric acid. The aqueous mixture is extracted with ethyl acetate and the organic phasis then treated as described above.
The limit of quantification of the method is 0.01 mg/kg in milk and 0.05 mg/kg in tissues for each analyte. The efficiency of the method was determined by fortifying control samples of each matrix with chloridazon and metabolite 4-hydroxy chloridazon. The average recovery in milk and tissues was 95 % for chloridazon and 90 % for 4-hydroxy chloridazon. In separated milk, the average recovery was found to be 104 % and 80 % for chloridazon and 4-hydroxy chloridazon, respectively.

Residues in whole milk

Mean results of each treatment group for both analytes are summarised in Table B.7.8-3 and Table B.7.8-4:

Table B.7.8-3: Summary of group mean results for whole milk (Groups I and II)

Treatment day	Group mean residues of chloridazon and BH 119-4-OH in whole milk [mg/kg]					
	Group I (Control)			Group II (1 x level)		
	Chloridazon	4-Hydroxy chloridazon[2]	Total residue[1]	Chloridazon	4-Hydroxy chloridazon[2]	Total residue[1]
-1	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02
1	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02
2	<0.01	<0.01	<0.02	n.a.	n.a.	n.a.
3	<0.01	<0.01	<0.02	n.a.	n.a.	n.a.
4	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02
5	<0.01	<0.01	<0.02	n.a.	n.a.	n.a.
7	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02
10	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02
13	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02
16	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02
19	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02
22	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02
25	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02
28	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02

[1]) For the calculation of the total residue, results below the LOQ (<0.01 mg/kg) have been given the value of the LOQ
[2]) Calculated in parent equivalents
n.a. not analysed

Table B.7.8-4: Summary of group mean results for whole milk (Groups III and IV)

Treatment day	Group mean residues of chloridazon and BH 119-4-OH in whole milk [mg/kg] [1]					
	Group III (3 x level)			Group IV (10 x level)		
	Chloridazon	4-Hydroxy chloridazon[2]	Total residue[1]	Chloridazon	4-Hydroxy chloridazon[2]	Total residue[1]
-1	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02
1	0.012	<0.01	0.022	0.029	<0.01	0.039
4	0.012	0.010	0.022	0.030	<0.01	0.040
7	0.011	<0.01	0.021	0.031	<0.01	0.041
10	0.025	<0.01	0.035	0.032	<0.01	0.042
13	0.023	<0.01	0.033	0.034	<0.01	0.044
16	0.017	<0.01	0.027	0.032	<0.01	0.042
19	0.018	<0.01	0.028	0.041	<0.01	0.051
22	0.014	<0.01	0.024	0.038	<0.01	0.048
25	0.012	<0.01	0.022	0.039	<0.01	0.049
28	0.011	<0.01	0.021	0.035	<0.01	0.045
29	n.a.	n.a.	n.a.	0.054	0.011	0.065
31	n.a.	n.a.	n.a.	<0.01	<0.01	<0.02
33	n.a.	n.a.	n.a.	<0.01	<0.01	<0.02

[1]) For the calculation of the total residue and average values, results below the LOQ (<0.01 mg/kg) have been given the value of the LOQ
[2]) Calculated in parent equivalents
n.a. not analysed

Residues in skim milk and cream

Two milk samples from animals of Group IV chosen for their high residue levels from study days 19 and 29 were separated into cream and skim milk. The results are summarised in Table B.7.8-5.

Table B.7.8-5: Summary of residues of chloridazon and 4-hydroxy chloridazon in separated milk samples from animals of Group IV (10 x Level)

Treatment day	Chloridazon [mg/kg]	4-Hydroxy chloridazon[2] [mg/kg]	Total residue[1] [mg/kg]
Cream			
19	0.075	<0.01	0.085
29	0.078	<0.01	0.088
Skim Milk			
19	0.060	<0.01	0.070
29	0.051	<0.01	0.061

[1]) For the calculation of the total residue, results below the LOQ (<0.01 mg/kg) have been given the value of the LOQ
[2]) Calculated in parent equivalents

Residues in tissues (kidney, liver, muscle and fat)

No residues of either chloridazon or 4-hydroxy chloridazon were detected in any tissues of the control group.

As well, no residues were detected in muscle tissue from the 1 x group and only small residues slightly above the LOQ were found in the 3 x dose group. Muscle tissue from the highest treatment group showed an average residue of chloridazon of 0.111 mg/kg, with individual values ranging from <0.05 mg/kg up to 0.205 mg/kg.

In fat tissues, residues slightly above the method's limit of quantification were found only in samples from the 10 x dose group.

In kidney samples from cows of the 1 x dose group no residues were detectable, whereas animals from the 3 x treatment group showed some residues of chloridazon (0.086 mg/kg) and in samples from the high dose group, residues of chloridazon and its hydroxyl metabolite were found.

Residues were measured in the liver samples of all three treatment groups. The amounts were increasing along with the dose rate and mean values ranged from 0.066 mg/kg for the 1x dose group to 0.167 mg/kg and 0.255 mg/kg in the middle and high dose group, respectively.

With the exception of the kidney samples of the 10 x dose level, no residues of metabolite 4-hydroxy chloridazon were found in any tissue type from all treated animals.

No residues were found in any of the tissues taken from the depuration cows.

The mean values of each treatment group are summarised in Table B.7.8-6.

Table B.7.8-6: Summary of group mean tissue results

Dose group	Matrix	Residue [mg/kg]		Total residue [mg/kg]
		Chloridazon	4-Hydroxy chloridazon	
I (Control)	Muscle	<0.05	<0.05	<0.10
	Fat	<0.05	<0.05	<0.10
	Liver	<0.05	<0.05	<0.10
	Kidney	<0.05	<0.05	<0.10
II (1x)	Muscle	<0.05	<0.05	<0.10
	Fat	<0.05	<0.05	<0.10
	Liver	0.066	<0.05	0.116
	Kidney	<0.05	<0.05	<0.10
III (3x)	Muscle	0.056	<0.05	0.106
	Fat	<0.05	<0.05	<0.10
	Liver	0.167	<0.05	0.217
	Kidney	0.086	<0.05	0.136
IV (10x)	Muscle	0.111	<0.05	0.161
	Fat	0.065	<0.05	0.115
	Liver	0.255	<0.05	0.305
	Kidney	0.176	0.059	0.235

Storage stability

All tissue samples of the cow feeding study were analysed within 2 months and the milk samples within 7 months after sampling. Thus, the deep freeze stability of chloridazon and metabolite 4-hydroxy chloridazon was investigated in milk and tissues (muscle and liver) over a period of 7 and 2 months, respectively. Samples of untreated cow milk, muscle and liver were fortified with chloridazon and 4-hydroxy chloridazon at 1.0 mg/kg each and stored under the usual storage conditions (in small plastic pots, below -15 °C). Fortified samples were analysed immediately (zero time point samples) and after storage intervals of 1 and 2 months (muscle and liver) or 3 and 7 months (milk). At each time point, one control sample, two stored fortification samples and two procedural recoveries (fortified with each analyte at 1 mg/kg) were analysed (see Table B.7.8-8). The procedural recoveries are summarised in Table B.7.8-7.

Table B.7.8-7: Procedural recoveries of chloridazon and BH 119-4-OH from milk and tissues[1] in % of the nominal fortification level

Storage Interval [Months]	Milk		Liver		Muscle	
	Chloridazon	4-Hydroxy chloridazon	Chloridazon	4-Hydroxy chloridazon	Chloridazon	4-Hydroxy chloridazon
0	77.4	88.6	75.2	90.8	97.0	90.2
1	n.a.	n.a.	79.6	85.9	89.6	80.3
2	n.a.	n.a.	93.2	75.3	91.1	76.6
3	86.0	90.0	n.a.	n.a.	n.a.	n.a.
7	80.2	85.3	n.a.	n.a.	n.a.	n.a.

[1]) Results represent mean values of two individual analyses
n.a.: not analysed

Table B.7.8-8: Storage recoveries of chloridazon and BH 119-4-OH from cow milk and tissues in percentage of the nominal fortification level (1.0 mg/kg)

Storage Interval [Months]	Milk		Liver		Muscle	
	Chloridazon	BH 119-4-OH	Chloridazon	BH 119-4-OH	Chloridazon	BH 119-4-OH
0	104.8	94.4	106.9	86.6	89.0	95.1
1	n.a.	n.a.	98.5	97.3	93.1	85.3
2	n.a.	n.a.	99.0	101.9	111.4	82.9
3	99.0	97.8	n.a.	n.a.	n.a.	n.a.
7	99.2	101.9	n.a.	n.a.	n.a.	n.a.

Results represent mean values of two individual analyses and results for stored fortifications are corrected for the procedural recoveries
n.a.: not analysed

Report: Weatherholtz W.M., 1970
28-day feeding and residue study - Dairy cows. Dephenylated pyrazon, pyrazon-N-glucoside,
[redacted]

Copeland G.L.,Stanovick R.P., 1970
Development of analytical methods and analysis for 1-phenyl-4-amino-5-chloro-6-pyridazone, 1-p-hydroxyphenyl-4-amino-5-chloro-6-pyridazone, 4-amino-5-chloro-6-pyridazone, and 1-phenyl-4-aminoglucosyl-5-chloro-6-pyridazone residues in samples of whole milk, muscle tissue, kidney tissue, liver tissue, and fat tissue from cows
[redacted]

GLP: no

Deviations: none

Acceptability: The study is considered to be acceptable.

Material and Methods:
Ten mature, lactating Holstein dairy cows in the weight range of 400 to 700 kg before treatment were used in the study. The test compounds were administered twice daily on 28 consecutive days at three dose levels. They were administered orally via an additional portion of feed (corn meal 'premix') mixed with the test substances. The animal of the control group received only basal diet.

Feeding and husbandry
The animals were housed in stanchions bedded on sawdust. They were allowed *ad libitum* access to a commercial milking chow via individual feeders and fresh tap water daily. Individual daily feed consumption was recorded during the study for each animal.

Selected dose levels and correlation with the estimated dietary burden
The cows were fed a diet containing the test items chloridazon metabolite A and metabolite B at a ratio of 3:7. The selected dose levels were 1 mg/kg (Group 2), 5 mg/kg (Group 3), and 20 mg/kg (Group 4) based on feed intake, corresponding to a nominal dosing of 0.7 mg/kg, 3.5 mg/kg and 14 mg/kg of chloridazon metabolite B.
To correlate these dose levels with the residues of metabolite B in animal feed that would now occur under normal agricultural practice, the dietary burden for different livestock species was calculated on the basis of current data (see Table B.7.8-9). The residue level for sugar beets is derived from the available residue trials and for grains from the rotational crop study.

Table B.7.8-9: Calculation of dietary burden of metabolite B for livestock

Feed item	% dry matter	% of diet	Residue level (HR)[1)]	Dietary burden		
			[mg/kg]	mg/animal/day	mg/kg bw	mg/kg feed
Dairy cattle						
Sugar beet leaves+tops	16	30	0.76	28.50	0.052	1.43
Grains	86	40	0.05	0.47	0.001	0.02
Sugar beets	20	30	0.10	3.00	0.005	0.15
Total diet:				31.97	0.058	1.60
Beef cattle						
Sugar beet leaves+tops	16	30	0.76	21.38	0.061	1.43
Grains	86	10	0.05	0.09	0.000	0.01
Sugar beets	20	60	0.10	4.50	0.013	0.30
Total diet:				25.96	0.074	1.73
Pigs						
Sugar beet leaves+tops	16	25	0.76	3.56	0.048	1.19
Grains	86	15	0.05	0.03	0.000	0.01
Sugar beets	20	60	0.10	0.90	0.012	0.30
Total diet:				4.49	0.060	1.50

[1]) HR = Highest residue value observed in trials

Dose preparation
The test items were incorporated into a 'premix' of corn meal at a ratio of 7:3 (metabolite B: metabolite A) at a level of 1000 mg/kg. The respective amount of this premix was weighed out daily for an estimated food consumption per cow based on its mean food consumption from the previous week and was hand-mixed into the milking chow diet.

Dose administration
Animals were dosed twice daily. The achieved daily intakes of metabolite A and metabolite B are listed in Table B.7.8-10. The actual doses were calculated on a mg/kg bodyweight, a mg/kg feed and a mg/animal/day basis, taking into account the averaged individual body weights in the beginning and at the end of the dosing period and the actual average individual feed intake (determined daily). After the dosing period of 28 days, two cows of each treatment group were

sacrificed (zero withdrawal). The third animal of each treatment group was maintained on a basal diet for a further two weeks (14 days withdrawal).

Table B.7.8-10: Dose groups with their nominal and actual dose levels

Dose group	Number of animals	Nominal dose Met. A /Met. B	Actual dose Metabolite A / Metabolite B		
		[mg/kg feed]	[mg/animal/day] [1)]	[mg/kg bw] [2)]	[mg/kg feed] [3)]
1	1	0	0	0	0
2	3	0.3/0.7	4.97/11.59	0.008/0.020	0.30/0.70
3	3	1.5/3.5	25.21/58.82	0.044/0.103	1.48/3.46
4	3	6.0/14.0	101.36/236.50	0.200/0.466	6.02/14.06

[1]) based on averaged individual daily doses
[2]) based on averaged individual body weights at the beginning and end of the study
[3]) based on averaged individual daily feed intake

Milking and milk sampling
All cows were milked twice daily with mechanical milkers and the individual total daily milk yield was recorded. The morning and evening milk samples of one day were pooled for each animal.
Duplicate milk samples were taken on study days 1, 3, 5, 8, 12, 18, 23 and 28 and on days 1, 3, 6 and 12 of the two weeks' withdrawal period (study days 29, 31, 34 and 40).
Concerning the mean milk production, occasional individual variations were considered to be within the normal limits and no treatment-related group differences were observed.

Terminal procedures
The animals were sacrificed by exsanguination following intravenous administration of Lethal Solution. Gross necropsies were performed and samples of muscle, liver, kidney, omental fat and blood (taken prior to sacrifice) were collected. The samples were sealed in airtight plastic bags and frozen until residue analysis.

Findings:
Bodyweight
About half of the cows showed a slight weight gain during the course of the experimental period whereas the remaining animals slightly lost weight. Bodyweight changes were considered to be within normal limits and no treatment-related group differences were observed.

Residue analysis
Samples were analysed for residues of chloridazon (which is formed from metabolite A, the N-glucoside of chloridazon), 4-hydroxy-chloridazon and chloridazon metabolite B.

Principle of the method:
Milk samples are extracted with a mixture of acetone/acetonitrile, simultaneously achieving a protein precipitation with the acetone. Tissues are mixed with sodium sulfate and also extracted with the acetone/acetonitrile mixture. Upon addition of water, the extracts are concentrated to the aqueous phase and divided for separate determination of chloridazon + 4-hydroxy-chloridazon and metabolite B.

Chloridazon + 4-hydroxy-chloridazon:
Extracts are directly subjected to an acid hydrolysis and then further purified by a partition with acetone/acetonitrile and chloroform. For final determination by GC-halide detection, the extract is derivatised with N,O-bis (trimethylsilyl)acetamide.

Chloridazon metabolite B:
The extract is purified by partitioning with hexane/chloroform followed by hexane and then subjected to the acid hydrolysis. After saturation with sodium chloride, the aqueous phase is partitioned with hexane and with acetone/acetonitrile/chloroform and final determination of the analyte is performed by GC combined with halide detection.

The following limits of quantification (LOQ) were achieved:

Chloridazon and 4-hydroxy-chloridazon:	0.1 mg/kg in milk and 0.3 mg/kg in tissues
Metabolite B:	0.1 mg/kg in milk and tissues

The efficiency of the method was determined by fortifying control samples of each matrix with chloridazon, 4-hydroxy-chloridazon and chloridazon metabolite B. The average recoveries by matrix were ranging from 80 % (muscle) to 100 %(liver) for chloridazon, from 75 % (muscle) to 97 % (liver) for 4-hydroxy-chloridazon and from 76 % (muscle) to 94 % (milk) for chloridazon metabolite B.

<u>Residues in whole milk</u>
The results of the analysis of whole milk samples are summarised in Table B.7.8-11.
No residues of chloridazon and its metabolite 4-hydroxy chloridazon above the LOQ were detected in whole milk from animals of the highest treatment group, which have received a dose of 20 mg/kg feed. Therefore, milk samples from animals of the 1 mg/kg and 5 mg/kg dose groups were not analysed for residues of chloridazon and 4-hydroxy-chloridazon. Analysis for metabolite B was performed with milk samples of all treatment groups. Residues above the LOQ were only found in samples of the 20 mg/kg dose group ranging between 0.21 mg/kg and 0.32 mg/kg with the highest individual value being 0.46 mg/kg. In milk samples from Groups 2 and 3 occasionally some residues of metabolite B were detected with group mean values from 0.007 mg/kg up to 0.078 mg/kg, but they were all below the LOQ.
No residues of chloridazon, 4-hydroxy-chloridazon or metabolite B were detected in whole milk from the control animal.
Milk samples drawn during the two weeks' depuration period were not analysed.

Table B.7.8-11: Summary of group mean results for whole milk

Treatment day	Group mean residues in whole milk [mg/kg]					
	Chloridazon	4-Hydroxy-chloridazon	Metabolite B	Chloridazon	4-Hydroxy-chloridazon	Metabolite B
	Group 1 (control)			Group 2 (dose level: 1 mg/kg)		
5	<0.1	<0.1	<0.1	n.a.	n.a.	n.a.
18	<0.1	<0.1	<0.1	n.a.	n.a.	n.a.
23	<0.1	<0.1	<0.1	n.a.	n.a.	<0.1 (0.021)*
28	<0.1	<0.1	<0.1	n.a.	n.a.	<0.1 (0.007)*
	Group 3 (dose level: 5 mg/kg)			Group 4 (dose level: 20 mg/kg)		
5	n.a.	n.a.	<0.1 (0.061)*	<0.1	<0.1	0.21
18	n.a.	n.a.	<0.1 (0.076)*	<0.1	<0.1	0.32
23	n.a.	n.a.	<0.1 (0.078)*	<0.1	<0.1	0.23
28	n.a.	n.a.	<0.1 (0.072)*	<0.1	<0.1	0.22

n.a. not analysed
*) the values in brackets give the amounts of metabolite B which were detected, but were below the limit of quantification

Residues in tissues (kidney, liver, muscle and fat)

No residues of chloridazon, metabolite 4-hydroxy-chloridazon or metabolite B above the LOQ were found in tissues of the control animal and of cows from Group 4 (dose level: 20 mg/kg).

Since tissues of the highest treatment group did not contain any residues, samples of the 1 mg/kg dose group were not analysed at all. Samples from the 5 mg/kg dose group were only analysed for metabolite B, but were also found to be free from any residues above the method's LOQ.

No tissues taken from the depuration cows were analysed.

Table B.7.8-12 gives an overview of the analysed tissues and the obtained results.

Table B.7.8-12: Summary of group mean tissue results

Matrix	Dose group	Residue [mg/kg]		
		Chloridazon	4-Hydroxy-chloridazon	Metabolite B
Muscle	1 (control)	<0.3	<0.3	<0.1
	3 (5 mg/kg)	n.a.	n.a.	<0.1
	4 (20 mg/kg)	<0.3	<0.3	n.a.
Fat	1 (control)	<0.3	<0.3	<0.1
	3 (5 mg/kg)	n.a.	n.a.	<0.1
	4 (20 mg/kg)	<0.3	<0.3	n.a.
Liver	1 (control)	<0.3	<0.3	<0.1
	3 (5 mg/kg)	n.a.	n.a.	<0.1
	4 (20 mg/kg)	<0.3	<0.3	n.a.
Kidney	1 (control)	<0.3	<0.3	<0.1
	3 (5 mg/kg)	n.a.	n.a.	<0.1
	4 (20 mg/kg)	<0.3	<0.3	n.a.

n.a. not analysed

Conclusion:

In the livestock feeding studies the amount of chloridazon and its metabolites reach a plateau in the second week of treatment. An accumulation of the substances could not be observed. Except one case all residues at 1x dosage level were below the LOQ in all analysed samples.

B.7.9 Residues in succeeding or rotational crops (Annex IIA 6.6; Annex IIIA 8.5)

Report: Ellenson J.L., 1993
Fate of ^{14}C-BAS 119 H in emergency, fall and annual replant confined rotational crops
BASF Corporation Agricultural Products Center, Research Triangle Park, NC 27709, USA

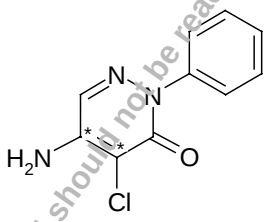
GLP: yes

Guideline: EPA Guideline Subdivision N, Series 165-1: Confined Rotational Crop

Deviations: none

Acceptability: The study is considered to be acceptable.

The residues of chloridazon (= Reg. No. 13033) in succeeding crops were investigated using ^{14}C-labelled test substance. The position of the label is shown below:



*: indicates position of ^{14}C label

Material and Methods:
The residue levels and the nature of the residues in three different succeeding crop groups (cereals: sorghum, wheat, oats; root and tuber: sugar beet; turnip and radish; leafy vegetables: chard) were investigated following application of ^{14}C-chloridazon. The investigations were performed under field- and greenhouse conditions.
For the field conditions the test substance was sprayed onto the ground. The entire plot was then sheltered with a plastic rain shield.

Rotational crops were grown in an outdoor plot in galvanised steel tanks (size: 90 x 60 x 60 cm) filled with a sand soil and treated at 3.47 kg as/ha with Pyramin DF herbicide fortified with ^{14}C-chloridazon. The following rotational crops were planted following treatment: chard and sorghum (39 DAT); chard and radish (103 DAT); wheat (144 DAT); sorghum (342 DAT); and chard and radishes (368 DAT).

For the greenhouse conditions, the test compound was taken up in methanol and mixed into a sandy soil at application rates of 3.36 kg as/ha and 8.85 kg as/ha. In the so called top-dressing method the soil premix was evenly dispersed over the untreated soil surface in each of the 18 plastic pots designated for the 3.36 kg as/ha treatment. In a similar manner 12 pots were treated with 8.85 kg as/ha.

The following rotational crops were planted in pots with treated soil at 3.36 kg as/ha: chard, sugar beets and wheat (29 and 89 DAT); chard, sugar beets and oats (266 DAT) and radishes and sugar beets (401 DAT). The following rotational crops were planted in soil treated at 8.86 kg as/ha: wheat and sugar beets (29 and 89 DAT); chard (99 DAT); chard, oats and sugar beets (266 DAT); and radishes and sugar beets (401 DAT).

Food and feed items of mature crops were harvested, processed and analysed by combustion/ extraction and subsequent radioactivity measurement for the determination of the total radioactive residues in the raw agricultural commodities (RAC's). In addition, soil samples were taken after application, after ploughing and after harvest of mature crops.
The total radioactive residues (TRR) of each sample were determined by direct combustion analysis and by adding up the extractable and the residual radioactivity values. If not stated otherwise, the values given below are calculated values.
Selected plant and soil samples were routinely extracted using a standard soxhlet extraction device. Methanol was used as the extraction solvent.
Plant samples having low moisture content, such as grain and fodder samples, were hydrated with water added to the samples prior to extraction. The methanol extract was concentrated and subjected to HPLC and/or TLC analysis.
Selected soxhlet-extracted residues were subjected to a subsequent hydrolysis by refluxing with 12 N HCL for 24 h. The solubilised extracts were concentrated and neutralised. Aliquots were applied to TLC plates for analysis.
The identification of metabolites is based on MS investigations and HPLC / TLC comparison with certified reference standards.
The soil characteristic is summarised in Table B.7.9-1.

Table B.7.9-1: Soil used to investigate radioactive residues in succeeding crops

Trial type	Field trial	Greenhouse trial
Origin of soil	Holly Springs, NC, BASF Field Test Site Plot 79	Metabolism Greenhouse. BASF ARC, RTP,NC
Soil type	Sand	Sand
% Organic matter	0.5	1.6
Textural analysis in %: Sand Silt Clay	 90 5 5	 91 4 5
Cation exchange capacity mVal/100 g	2.3	4.8
Soil pH	6.5	4.9

Findings:
The distribution of the total radioactive residues (TRR), the extractable radioactive residues (ERR) and the residual radioactive residues (RRR) in the individual samples are summarised in Table B.7.9-2 and Table B.7.9-3. Total ^{14}C-residues of at least 0.4 mg/kg (chloridazon equivalents) were found in all rotational crops. Soxhlet/methanol extraction was performed on immature and mature crop parts.

Table B.7.9-2: Quantitative distribution of radioactive residues in rotational crops after treatment with ^{14}C-chloridazon (greenhouse)

Sample material	Application rate [kg/ha]	Replant interval [DAT]	Harvest [DAP]	Total [DAT]	TRR [mg/kg]	% TRR in MeOH extract	% TRR in residue [RRR]
Chard	8.85	89	133	222	7.9	77	23
Chard	8.85	266	132	398	8.2	87	13
Sugar beet Tops	8.85	89	133	222	10.6	75	25
Sugar beet Tops	8.85	266	166	432	3.3	96	4
Sugar beet roots	8.85	89	133	222	2.3	50	50
Sugar beet roots	8.85	266	166	432	0.6	41	59
Oat forage	3.36	266	48	314	5.9*	n.p.	n.p.
Oat forage	8.85	266	50	316	10.6	91	9
Oat Fodder	3.36	266	117	383	12.2	n.p.	n.p.
Oat Fodder	8.85	266	120	386	23.0	88	12
Oat Grain	3.36	266	117	383	3.8	n.p.	n.p.
Oat Grain	8.85	266	120	386	20.0	75	25

* determined by direct combustion analysis
n.p.: not performed
DAT: days after treatment
DAP: days after planting

Table B.7.9-3: Quantitative distribution of radioactive residues in rotational crops after treatment with ^{14}C-chloridazon (field trials)

Sample material	Application rate [kg as/ha]	Planting [DAT]	Harvest [DAP]	Total [DAT]	TRR [mg/kg]	% TRR in MeOH extract	% TRR in residue [RRR]
Chard	3.47	39	63	102	11.2*	n.p.	n.p.
Chard	3.47	103	41; 55	144;158	5.6*	n.p.	n.p.
Chard	3.47	368	29	397	2.7	97	3
Radish Tops	3.47	103	41; 55; 71	144;158;174	2.6*	n.p.	n.p.
Radish Tops	3.47	368	29	397	2.2	93	7
Radish Roots	3.47	103	41; 55; 71	144;158;174	0.3	78	22
Radish Roots	3.47	368	29	397	0.4*	n.p.	n.p.
Sorghum Fodder	3.47	39	63	102	5.9	81	19
Sorghum Forage	3.47	342	78	420	2.5*	n.p.	n.p.
Sorghum Forage	3.47	39	105	144	2.2	99	1
Sorghum Fodder	3.47	342	104	446	17.7*	n.p.	n.p.
Sorghum Grain	3.47	39	105	144	1.6	90	10
Sorghum Grain	3.47	342	104	446	0.4*	n.p.	n.p.
Wheat Forage	3.47	144	114	258	4.1	67	33
Wheat Fodder	3.47	144	224	368	11.8	89	11
Wheat Grain	3.47	144	244	368	2.4	78	22

*) determined by direct combustion analysis
n.p.: not performed
DAT: Days After Treatment
DAP: Days After Planting

The results of the combustion analyses of soil are shown in Table B.7.9-4.

Table B.7.9-4: Total radioactive residues in soil samples after treatment with 14C-chloridazon (field test site)

Soil samples [DAT]	Soil cores		
	0 - 15 cm [mg/kg]	15 - 30 cm [mg/kg]	> 30 cm [mg/kg]
Pre application	0.00	--	---
Post application	4.07	--	---
39	0.53	0.04	0.00
102	0.81	0.06	0.01
102	0.76	0.02	---
258	0.68	0.04	0.02
342	0.62	0.04	0.05
368	0.92	0.16	0.01
397	1.00	0.22	0.04
397	1.15	0.12	0.03
446	0.31	0.08	0.01

For the greenhouse portion of the study, total radioactive residues (TRR) in the soil were determined prior to dispensing into pots and at subsequent sampling intervals. These TRR values were determined by oxidative combustion of aliquots to $^{14}CO_2$ and liquid scintillation counting (LSC). The results are shown in Table B.7.9-5:

Table B.7.9-5: Total radioactive residues in soil samples after treatment with 14C-chloridazon (greenhouse)

Soil samples [DAT]	Rate: 8.86 kg/ha [mg/kg]	Rate: 3.36 kg/ha [mg/kg]
Pre application	0.0	0.0
Post application	s.n.c.	s.n.c.
29	11.7	6.1
89	13.9	4.9
99	10.5	s.n.c.
120	s.n.c.	3.6
222	3.9	1.2
253	3.7	s.n.c.
314	2.4	1.6
383	2.5	0.9
398	2.0	s.n.c.
432	1.4	s.n.c.

s.n.c. = samples not collected

High performance liquid chromatographic (HPLC) analyses were performed on the methanol extracts for the identification and quantification of chloridazon and its metabolites. The results of quantification are shown in Table B.7.9-6 and Table B.7.9-7.

Another major metabolite (P3) was tentatively identified as an analog of metabolite B and ranged from 0.01 mg/kg in sugar beet roots (annual replants) to 5.41 mg/kg in oat fodder. This was the only major unidentified metabolite which represented a maximum of about 20 % of the total extractable residues (oat fodder, but only when grown indoors under forced uptake conditions 8.86 kg as/ha. However, when grown in the outdoor confined plot with an application rate of 3.48 kg as/ha, which is still higher than the intended 2.6 kg as/ha, residue levels of P3 were less than 5 %.

Lesser amounts of two metabolites, the 4-hydroxy analog of chloridazon and an unidentified metabolite (P5) were found. The 4-hydroxy analog of chloridazon ranged from 0.01 mg/kg in sugar beet roots (annual replants) to 0.48 mg/kg in oat grain, and metabolite P5 ranged from 0.01 mg/kg in sugar beet roots (annual replants) to 1.00 mg/kg in oat grain. In addition, residues to two polar metabolites (P1 and P2) were present. Metabolite P1 ranged from 0.02 mg/kg in sugar beet tops (annual replants) to 2.0 mg/kg in oat grain, and metabolite P2 ranged from 0.03 mg/kg in sugar beet roots to 1.28 mg/kg in sugar beet tops (fall replants).

Table B.7.9-6: Investigation of the nature of the residues: Summary of major components in rotational crops after treatment with ^{14}C-chloridazon and plant back intervals of 39 and 144 days (field trials)

Crop parts (DAP)	Rate [kg as/ha]	TRR [mg/kg]	MeOH mg/kg [% TRR]	Parent (P7) [mg/kg] (% TRR)	Metab. B (P4) [mg/kg] (% TRR)	Metabolites [mg/kg] (% TRR)
Plant back interval: 39 DAT						
Sorghum forage (63)	3.47	2.2	99	0.77 (35.3)	0.26 (11.8)	Polar metabolite (P1):0.23 (10.4 %) Polar metabolite (P2): 0.05 (2.4 %) Isomer of met. B (P3): 0.02 (0.7 %) unknown (P5): 0.03 (1.3 %) p-OH-chloridazon: (P6): 0.14 (6.6 %)
Sorghum fodder (105)	3.47	5.9	81	1.63 (27.6)	1.62 (27.4)	Polar metabolite (P1): 0.12 (2.0 %) Polar metabolite (P2): 0.11 (1.9 %) Isomer of met. B (P3): 0.12 (2.0 %) unknown (P5): 0.11 (1.9 %) p-OH-chloridazon (P6): 0.47 (8.0 %)
Sorghum grain (105)	3.47	1.6	90	0.41 (26.1)	0.84 (54.3)	Polar metabolite (P1): 0.05 (3.1 %) Polar metabolite (P2): 0.07 (4.6 %) Isomer of met. B (P3): --- unknown (P5): 0.02 (1.0 %) p-OH-chloridazon (P6): 0.04 (2.4 %)
Plant back interval: 144 DAT						
Wheat forage (114)	3.47	4.1	89	0.80 (19.3)	1.90 (46.0)	Polar metabolite (P1): 0.39 (9.5 %) Polar metabolite (P2): 0.14 (3.4 %) Isomer of met. B (P3): 0.11(2.7 %) Unknown (P5): ------ p-OH-chloridazon (P6): 0.27 (6.6 %)
Wheat fodder (224)	3.47	11.8	67	0.86 (7.3)	6.12 (52.0)	Polar metabolite (P1): 0.44 (3.7 %) Polar metabolite (P2): 0.40 (3.4 %) Isomer of met. B (P3): 0.39 (3.3 %) Unknown (P5): ------- p–OH-chloridazon (P6): -----
Wheat grain (224)	3.47	2.4	78	0.15 (6.3)	1.46 (60.4)	Polar metabolite (P1): 0.09 (3.9 %) Polar metabolite (P2): 0.04 (1.8 %) Isomer of met. B(P3):0.11 (4.6 %) Unknown (P5): ------ p-OH-chloridazon (P6): ------

Table B.7.9-7: Investigation of the nature of the residues: Summary of major components in rotational crops after treatment with ^{14}C-chloridazon and plant back intervals of 89, 99 and 266 days (greenhouse)

Crop parts (DAP)	Rate [kg as/ha]	TRR [mg/kg]	MeOH mg/kg [% TRR]	Parent (P7) [mg/kg] (% TRR)	Metab. B (P4) [mg/kg] (% TRR)	Metabolites [mg/kg] (% TRR)
Plant back interval: 89 DAT						
S. beet tops (133)	8.85	10.6	75	---	6.95 (65.5)	Polar metabolite (P1): 0.31 (2.9 %) Polar metabolite (P2): 1.28 (12.1 %) Isomer of met. B (P3): 0.88 (8.3 %) unknown (P5): --- p - OH-chloridazon (P6): ---
S. beet roots (133)	8.85	2.3	50	0.17 (7.4)	0.96 (42.5)	Polar metabolite (P1): 0.27 (11.9 %) Polar metabolite (P2): 0.15 (6.5 %) Isomer of met. B (P3): 0.13 (5.7 %) unknown (P5): --- p-OH-chloridazon (P6): 0.11 (5.0 %)
Plant back interval: 99 DAT						
Chard plant (123)	8.85	7.9	77	0.08 (1.0)	6.28 (79.3)	Polar metabolite (P1): 0.21 (2.6 %) Polar metabolite (P2): 0.25 (3.1 %) Isomer of met B (P3): 0.80 (10.1 %) unknown (P5): --- p-OH-chloridazon (P6): 0.03 (0.4 %)
Plant back interval: 266 DAT						
S. beet root (166)	8.85	0.6	41	0.02 (3.0)	0.26 (44.2)	Polar metabolite (P1): 0.12 (21.1 %) Polar metabolite (P2): 0.03 (5.6 %) Isomer of met. B (P3): 0.01 (1.5 %) Unknown (P5): 0.01 (2.3 %) p-OH-chloridazon (P6): 0.01 (1.5 %)
S. beet tops (166)	8.85	3.3	96	--- (---)	2.76 (83.3)	Polar metabolite (P1): 0.02 (0.6 %) Polar metabolite (P2): 0.08 (2.3 %) Isomer of met. B (P3): 0.13 (3.9 %) Unknown (P5): ------ p-OH-chloridazon (P6): ----
Chard plant (132)	8.85	8.2	87	--- (---)	6.09 (74.2)	Polar metabolite (P1): 0.16 (2.0 %) Polar metabolite (P2): 0.10 (1.2 %) Isomer of met. B (P3): 0.78 (9.5 %) Unknown (P5): 0.11 (1.3 %) p-OH-chloridazon (P6): ----
Oat forage (50)	8.85	10.6	91	0.01 (0.1)	7.79 (73.4)	Polar metabolite (P1): 0.30 (2.8 %) Polar metabolite (P2): 0.17 (1.6 %) Isomer of met. B (P3): 1.71 (16.1 %) Unknown (P5): 0.09 (0.8 %) p-OH-chloridazon (P6): 0.03 (0.3 %)
Oat fodder (120)	8.85	23.0	88	0.30 (1.3)	11.07 (48.1)	Polar metabolite (P1): 0.85 (3.7 %) Polar metabolite (P2): 0.58 (2.5 %) Isomer of met. B (P3): 5.41(23.5 %) unknown (P5): 0.53 (2.3 %) p-OH-chloridazon (P6): 0.41 (1.8 %)

Oat grain (120)	8.85	20.0	75	--- (---)	9.67 (48.4)	Polar metabolite (P1): 2.00 (10.0 %) Polar metabolite (P2): 0.54 (2.7 %) Isomer of met. B (P3): 2.46 (12.3 %) unknown (P5): 1.00 (5.0 %) p-OH-chloridazon (P6): 0.48 (2.4 %)

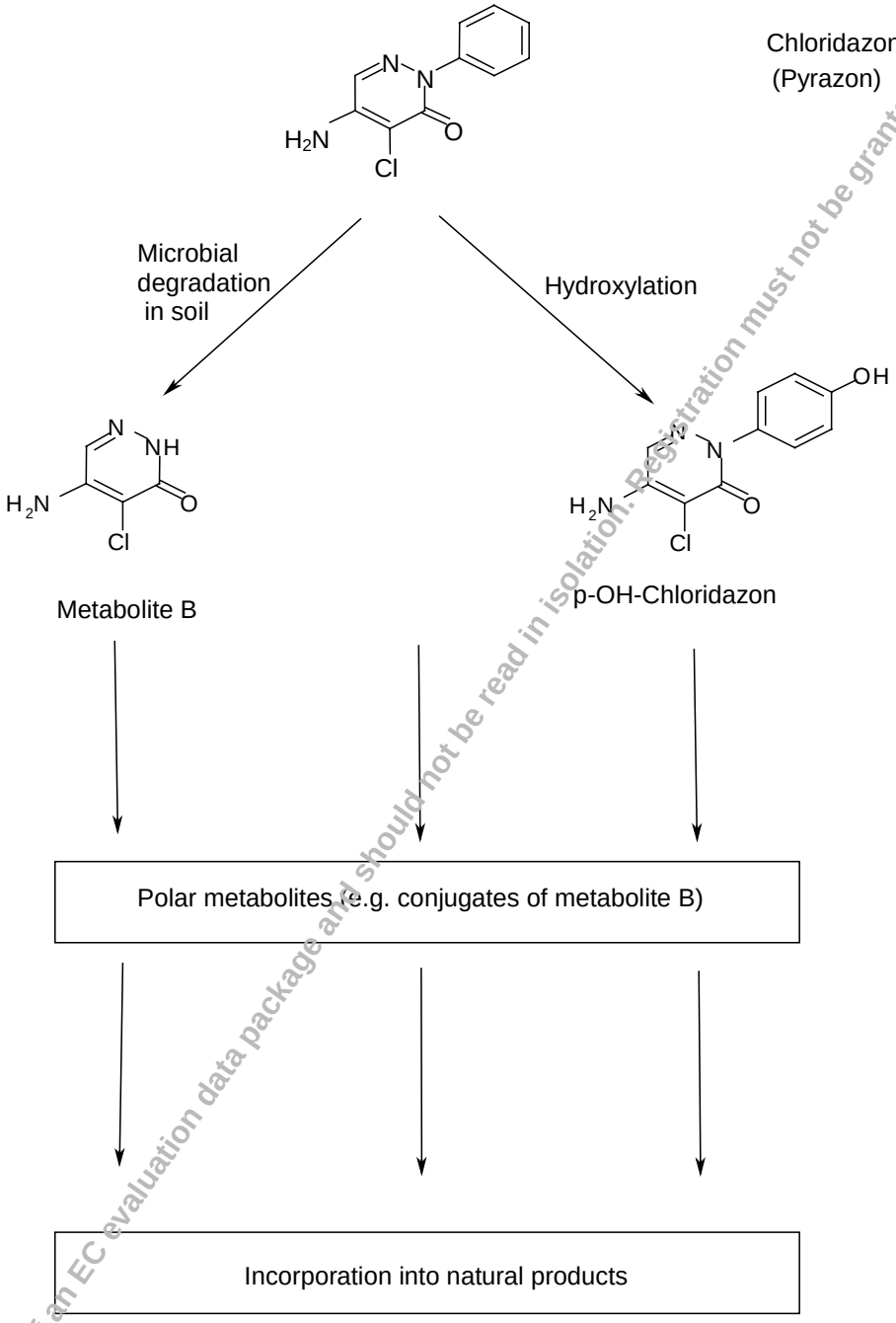
Acid hydrolysis of selected soxhlet-extracted residues of plant and soil samples led to the additional release of maximum 28 % of the TRR (89 DAT greenhouse sugar beet roots), see Table B.7.9-8. The radioactivity released from plants mainly consisted of metabolite B plus some non-polar components that showed a comparable chromatographic behaviour than the parent molecule.

Table B.7.9-8: Investigation of the nature of the residues: Further characterisation of the non-released radioactivity in rotational crops after treatment with ^{14}C-chloridazon and plant back intervals of 39, 144 days (field conditions) and 89, 99 and 266 days (greenhouse conditions)

Sample Material (DAP)	Appl. Rate [kg as/ha]	% TRR in residues after solvent extraction	% TRR released by hydrolysis	% TRR in the final residue
Plant back interval: 39 DAT (field)				
Sorghum forage (63)	3.47	1	n.r.	n.r.
Sorghum fodder (105)	3.47	19	9	10
Sorghum grain (105)	3.47	10	4	5
Plant back interval: 144 DAT (field)				
Wheat forage (114)	3.47	11	7	4
Wheat fodder (224)	3.47	33	11	22
Wheat grain (224)	3.47	22	4	19
Plant back interval: 89 DAT				
S. beet tops (133)	8.86	25	14	11
S. beet roots (133)	8.86	50	28	22
Plant back interval: 99 DAT				
Chard plant (123)	8.86	23	10	14
Plant back interval: 266 DAT				
S. beet root (166)	8.86	59	21	38
S. beet tops (166)	8.86	4	2	2
Chard plant (132)	8.86	13	2	11
Oat forage (50)	8.86	9	6	3
Oat grain (120)	8.86	25	19	6

n.r. = not reported: Hydrolysis data for sorghum forage were suspect 20-fold in excess recovery of an approx. Radioactivity found in the hydrolysis extract indicated that some error had occurred during hydrolysis

Figure B.7.9-1: Proposed metabolic pathway of chloridazon in rotational crops



Conclusion:

In the studies it could be shown, that residues above the limit of quantification may occur in succeeding crops after the pre-emergence application of chloridazon. In the field trials the application rate was approximately 1.5x of the intended use. In succeeding cereals, the residue levels exceeded 1.0 mg/kg in grain and went up to 11.8 mg/kg in wheat fodder.

The results of the greenhouse trials with an application rate of 8.86 kg as/ha confirm an massive uptake of the parent substance and the soil metabolite B.

Based on these studies it can not be guaranteed that no residues above the LOQ may occur in succeeding crops.

Report: Zehr R.D., 1996(b)
Pyramin herbicide limited field rotational crop study
BASF Corporation Agricultural Products Center, Research Triangle Park, NC 27709, USA

GLP: yes

Guideline: EPA Guideline Subdivision N, 165-2: Field Rotational Crops

Deviations: none

Acceptability: The study is considered to be acceptable.

Material and Methods:
The purpose of this study was to determine the potential for selected field rotational crops including spring wheat, leaf lettuce and potatoes to take up soil residues of chloridazon and its soil metabolite ("metabolite B") at replant interval of one year.
A total of 6 trials were run in the USA, in the states: California, Michigan and North Dakota. At each trial, there was one control and one treated plot. The treated plot received two sequential banded applications of Pyramin DF Herbicide, including one pre emergence and one post emergence application applied over bare ground. The target rate for the pre emergence application was 6.7 kg as/ha, the post emergence rate was 1.79 kg as/ha. Approximately 360 days after the second treatment, rotational crops were planted: spring wheat was planted at two sites in North Dakota; leaf lettuce, at two sites in California; and potatoes, at two sites in Michigan.
Two replicates of each matrix from each site were analysed by modified versions of BASF Method A9202. The analytes were chloridazon and its dephenylated metabolite (metabolite B).
Control rotational crop samples fortified with 0.05 mg/kg and 1.0 mg/kg each of chloridazon and metabolite B were analysed concurrently with the treated samples. Mean method recoveries overall averaged 94 $\pm$ 18 % (N=18) for chloridazon and 80 $\pm$ 11 % (N=19) for metabolite B.

Findings:
Results were expressed in terms of total residues of chloridazon. The highest total residues detected were in leaf lettuce samples from the treated plot from one of the California sites: 0.293-0.299 mg/kg. The residues in leaf lettuce from the other California site were less than 0.1 mg/kg (the limit of quantification). Other quantifiable residues were found in spring wheat forage: 0.149 to 0.184 mg/kg in North Dakota in one site; 0.141 and $\leq$0.1 mg/kg in the other site; results in spring wheat hay varied from site to site: from <0.1 mg/kg to 0.140 mg/kg; and potato: <0.10 to 0.111 mg/kg in Michigan. No residues greater than the limit of quantification (0.1 mg/kg) were found in any of the samples of wheat grain, or wheat straw. No residues greater than the limit of quantification were found in any of the control plots from these sites.

All residue data are summarised in Table B.7.9-9.

Table B.7.9-9: Summary of residues of chloridazon in rotational crops

Crop	Matrix	State	Days, Application to Planting	Days, Application to Harvest	Total Residue Chloridazon Equivalents[1] [mg/kg]
Spring Wheat	Forage	North Dakota	360	384	0.149, 0.184
		North Dakota	360	386	<0.10, 0.141
	Hay	North Dakota	360	424	0.117, 0.140
		North Dakota	360	424	<0.10, <0.10
	Grain	North Dakota	360	468	<0.10, <0.10
		North Dakota	360	468	<0.10, <0.10
	Straw	North Dakota	360	468	<0.10, <0.10
		North Dakota	360	468	<0.10, <0.10
Lettuce	Leaves	California	360	409	0.293, 0.299
		California	360	434	<0.10, <0.10
Potato	Tubers	Michigan	369	486	<0.10, 0.111
		Michigan	356	495	<0.10, <0.10

[1]) The total residue is the sum of the mg/kg values (in parent equivalents) for chloridazon and metabolite B.
Application Rate: 8.52 kg as/ha [6.73 kg as/ha + 1.79 kg as/ha]

Storage stability information generated in this study indicates residues of metabolite B were stable for at least 8 months in lettuce. Residues of chloridazon and metabolite B were stable for 7 months in fortified wheat grain and fortified wheat straw.

Conclusion:
In this study the residues in succeeding crops were under the limit of quantification in most cases. Only lettuce and forage/hay of spring wheat showed residues up to 0.3 mg/kg. The crops were all planted 360 days after the last application and harvested 400 to 500 days later. Under consideration of the longer interval between application and harvest this study confirms the possibility that residues above the LOQ may occur in succeeding crops.

B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods, in the case of post-harvest uses (Annex IIA 6.8; Annex IIIA 8.7)

Chloridazon is used as an pre-emergence and post-emergence herbicide. Any pre-harvest interval is not considered necessary, since it is cover by the stage of growth of the treated crops.

B.7.11 Community MRLs and MRLs in EU Member States (Annex IIIA 12.2)

International Organisation or Country / EU Member State	Commodity	MRL [mg/kg]	Residue Definition
Austria	Sugar beet	0.5	Parent and metabolite B (expressed as chloridazon)
	Red beet, carrot, Swiss chard	0.3	Parent and metabolite B (expressed as chloridazon)
	Other vegetable food	0.1	Parent and metabolite B (expressed as chloridazon)
Belgium	Vegetables	0.1	Parent
	Sugar beet, fodder beet	0.05	Parent
Germany	Sugar beet, red beet, Swiss chard	0.5	Parent and metabolite B
	Other vegetable food	0.1	Parent and metabolite B
Italy	Bulb vegetables, sugar beet, fodder beet, red beet	0.1	Parent
Luxembourg	Red beet	0.5	Parent
	Other vegetable food	0.1	Parent
Netherlands	Red beet, other food/ feedstuffs of plant origin	0.05	Parent
Spain	Fodder beet	0.5	Parent
	Sugar beet, Swiss chard, spinach	0.1	Parent
	Other food/ feedstuffs of plant origin	0.05	Parent

B.7.12 Proposed EU MRLs and justification for the acceptability of those residues (Annex IIA 6.7; Annex IIIA 8.6)

B.7.12.1 Plant matrices

The submitted supervised trials for sugar and red beet were analysed for the sum of chloridazon and metabolite A. The residue definition for enforcement purposes was proposed without metabolite A. Therefore, the calculated MRLs result in an overestimation. Even with the higher MRLs a risk for the consumer is unlikely.

B.7.12.1.1 Sugar beet

In 24 trials (in 1975 to 1977 in Germany and in 2000 to 2001 in Belgium, France, Italy and Spain) the following total residues of chloridazon, metabolite A and metabolite B were found in sugar beet roots:

Residues in sugar beet roots after 98 to 183 days:
<0.13(13), 0.13(7), 0.14, 0.16, 0.18, 0.20 mg/kg chloridazon/metabolite A + metabolite B, calculated as chloridazon

STMR: 0.13 mg/kg
HR: 0.20 mg/kg

The methods described in 7039/VI/95 EN of 22.07.97 were used for deriving the MRL proposal.

Table B.7.12-1: MRL calculation for sugar beets

	Total in mg/kg
Method 1	0.178
Method 2	0.260

As a conclusion the following MRL is proposed:
0.3 mg/kg for total residues of chloridazon and metabolite B in sugar beet.

B.7.12.1.2 Red beets

In the six trials performed in 1978 in Germany, the following total residues of chloridazon, metabolite A and metabolite B were found in red beet roots:

Residues in red beet roots after 93 days:
<0.13, 0.14, 0.16(3), 0.19 mg/kg chloridazon/metabolite A + metabolite B, calculated as chloridazon

STMR: 0.16 mg/kg
HR: 0.19 mg/kg

The methods described in 7039/VI/95 EN of 22.07.97 were used for deriving the MRL proposal.

Table B.7.12-2: MRL calculation for red beets

	Total in mg/kg
Method 1	0.233
Method 2	0.335

As a conclusion the following MRL is proposed:
0.5 mg/kg for total residues of chloridazon and metabolite B in red beets.

B.7.12.1.3 Chard

Residue data for beet leafs and tops can be used for the calculation of MRLs. As a critical case residue data for red beet leafs and tops are used.

Residues in red beet tops after 93 days:
0.62, 0.76, 0.86, 1.2, 1.4, 1.5 mg/kg chloridazon/metabolite A + metabolite B, calculated as chloridazon

STMR: 1.03 mg/kg HR: 1.5 mg/kg

The methods described in 7039/VI/95 EN of 22.07.97 were used for deriving the MRL proposal.

Table B.7.12-3: MRL calculation for chard

	Total in mg/kg
Method 1	2.397
Method 2	2.850

As a conclusion the following MRL is proposed:
3 mg/kg for total residues of chloridazon and metabolite B in chard.

B.7.12.1.4 Onions

In eight trials (in 2000 in Germany and Great Britain and in 2001 in Belgium, Denmark and Sweden) the following total residues of chloridazon, metabolite A and metabolite B were found in onion bulbs:

Residues in onion bulbs after 78 to 122 days:
<0.13(3), 0.14 mg/kg chloridazon/metabolite A + metabolite B, calculated as chloridazon
STMR: 0.13 mg/kg
HR: 0.14 mg/kg

Residues in onion bulbs after 108 to 146 days:
<0.13(4) mg/kg chloridazon + metabolite B, calculated as chloridazon
STMR: 0.13 mg/kg
HR: 0.13 mg/kg

The methods described in 7039/VI/95 EN of 22.07.97 were used for deriving the MRL proposal. Therefore, the residue values were combined.

Table B.7.12-4: MRL calculation for onions

	Total in mg/kg
Method 1	0.130
Method 2	0.260

As a conclusion the following MRL is proposed:
0.2 mg/kg for total residues of chloridazon and metabolite B in onion.

B.7.12.1.5 Justification of acceptability

The proposed MRLs cover the residues, which might occur after the use of chloridazon containing pesticides according to intended uses. The estimation of the acute and chronic dietary consumer risk shows that there is no risk for consumers.

B.7.12.2 Products of animal origin

Chloridazon + metabolite A:
Based on the supervised trials the following residues were found in feeding stuff:
Chloridazon + metabolite A, calculated as chloridazon:
Sugar beet: tops / leaves + tops:
< 0.05(22), 0.06, 0.08 mg/kg
STMR: 0.05 mg/kg HR: 0.08 mg/kg
Roots:
< 0.05(23), 0.06 mg/kg

In the livestock feeding studies the residues in animal matrices reached a plateau in the second week. Therefore, the highest residues (HR) are used for the calculation of the dietary burden (see Table B.7.12-5).

Table B.7.12-5 Feed intake calculation: chloridazon + metabolite A

	% DM dry matter	Chicken	Dairy Cattle	Beef Cattle	Pig	Residue (mg/kg)	Intake (mg/kg dry feed basis)			
							Chicken	Dairy Cattle	Beef Cattle	Pig
Body weight		1,9 kg	550 kg	350 kg	75 kg					
Daily Maximum Feed (Dry Matter (DM))		120 g	20 kg	15 kg	3 kg					
Maximum Percentage		% DM	% DM	% DM	% DM					
I. Green Forage (incl. Hay)										
Grasses	20.000	0.000	100.000	100.000	0.000		0.000	0.000	0.000	0.000
Alfalfa/Clover	20.000	0.000	40.000	40.000	15.000		0.000	0.000	0.000	0.000
Forage Rape	14.000	0.000	0.000	35.000	15.000		0.000	0.000	0.000	0.000
Kale/Cabbage	14.000	5.000	35.000	35.000	15.000		0.000	0.000	0.000	0.000
Sugar Beet leaves and tops	16.000	0.000	30.000	30.000	25.000	0.080	0.000	0.150	0.150	0.125
Silage (Clover, Grasses, Vines of	20.000	0.000	100.000	100.000	15.000		0.000	0.000	0.000	0.000
Legumes)										
Fruit Pomace (Apples, Citrus, Grape)	23.000	0.000	10.000	30.000	0.000		0.000	0.000	0.000	0.000
Hay	85.000	0.000	100.000	100.000	15.000		0.000	0.000	0.000	0.000
II. Grains										
Grains except Maize	86.000	70.000	40.000	80.000	80.000		0.000	0.000	0.000	0.000
Maize	86.000	70.000	30.000	30.000	40.000		0.000	0.000	0.000	0.000
Bran (Wheat and Rye)	89.000	15.000	20.000	20.000	20.000		0.000	0.000	0.000	0.000
III. Straw Cereals)	86.000	0.000	20.000	50.000	0.000		0.000	0.000	0.000	0.000
IV. Pulses	86.000	30.000	20.000	20.000	40.000		0.000	0.000	0.000	0.000
V. Root and Tubers (e.g. Potatoes)	15.000	20.000	30.000	60.000	60.000	0.060	0.080	0.120	0.240	0.240
Swede/Turnip	10.000	20.000	30.000	60.000	60.000		0.000	0.000	0.000	0.000
Sugar and Fodder Beet	20.000	20.000	30.000	60.000	60.000	0.060	0.060	0.090	0.180	0.180
VI. Oil Seed (Meal, Cake)	86.000	10.000	30.000	30.000	20.000		0.000	0.000	0.000	0.000
(e.g. Soya bean, Peanuts, Rape seed,										
Sunflower seed, Linseed)										
Maximum intake (mg/kg feed)							**0.080**	**0.270**	**0.390**	**0.365**
Maximum intake (mg/kg bw)							**0.005**	**0.010**	**0.017**	**0.015**
Maximum intake (mg/animal)							**0.010**	**5.400**	**5.850**	**1.095**

Metabolite B:

Tops / leaves + tops:
<0.05(3), 0.07, 0.1, 0.1, 0.13, 0.13, 0.17, 0.18, 0.22, 0.22, 0.23, 0.30, 0.30, 0.31, 0.36, 0.38, 0.39, 0.39, 0.42, 0.48, 0.48, 0.52 mg/kg
STMR: 0.225 mg/kg
HR: 0.52 mg/kg

Roots:
<0.05(13), 0.05(7), 0.06, 0.07, 0.08, 0.1 mg/kg
STMR: 0.05 mg/kg
HR: 0.1 mg/kg

In the livestock feeding studies the residues in animal matrices reached a plateau in the second week. Therefore, the highest residues (HR) are used for the calculation of the dietary burden
(see Table B.7.12-6).

Table B.7.12-6 Feed intake calculation: metabolite B

	% DM dry matter	Chicken	Dairy Cattle	Beef Cattle	Pig	Residue (mg/kg)	Intake (mg/kg dry feed basis)			
							Chicken	Dairy Cattle	Beef Cattle	Pig
Body weight		1,9 kg	550 kg	350 kg	75 kg					
Daily Maximum Feed (Dry Matter (DM))		120 g	20 kg	15 kg	3 kg					
Maximum Percentage		% DM	% DM	% DM	% DM					
I. Green Forage (incl. Hay)										
Grasses	20.000	0.000	100.000	100.000	0.000		0.000	0.000	0.000	0.000
Alfalfa/Clover	20.000	0.000	40.000	40.000	15.000		0.000	0.000	0.000	0.000
Forage Rape	14.000	0.000	0.000	35.000	15.000		0.000	0.000	0.000	0.000
Kale/Cabbage	14.000	5.000	35.000	35.000	15.000		0.000	0.000	0.000	0.000
Sugar Beet leaves and tops	16.000	0.000	30.000	30.000	25.000	0.520	0.000	0.975	0.975	0.813
Silage (Clover, Grasses, Vines of Legumes)	20.000	0.000	100.000	100.000	15.000		0.000	0.000	0.000	0.000
Fruit Pomace (Apples, Citrus, Grape)	23.000	0.000	10.000	30.000	0.000		0.000	0.000	0.000	0.000
Hay	85.000	0.000	100.000	100.000	15.000		0.000	0.000	0.000	0.000
II. Grains										
Grains except Maize	86.000	70.000	40.000	80.000	80.000		0.000	0.000	0.000	0.000
Maize	86.000	70.000	30.000	30.000	40.000		0.000	0.000	0.000	0.000
Bran (Wheat and Rye)	89.000	15.000	20.000	20.000	20.000		0.000	0.000	0.000	0.000
III. Straw Cereals)	86.000	0.000	20.000	50.000	0.000		0.000	0.000	0.000	0.000
IV. Pulses	86.000	30.000	20.000	20.000	40.000		0.000	0.000	0.000	0.000
V. Root and Tubers (e.g. Potatoes)	15.000	20.000	30.000	60.000	60.000		0.000	0.000	0.000	0.000
Swede/Turnip	10.000	20.000	30.000	60.000	60.000	0.100	0.200	0.300	0.600	0.600
Sugar and Fodder Beet	20.000	20.000	30.000	60.000	60.000	0.100	0.100	0.150	0.300	0.300
VI. Oil Seed (Meal, Cake)	86.000	10.000	30.000	30.000	20.000		0.000	0.000	0.000	0.000
(e.g. Soya bean, Peanuts, Rape seed, Sunflower seed, Linseed)										
Maximum intake (mg/kg feed)							**0.200**	**1.275**	**1.575**	**1.413**
Maximum intake (mg/kg bw)							**0.013**	**0.046**	**0.068**	**0.057**
Maximum intake (mg/animal)							**0.024**	**25.500**	**23.625**	**4.238**

For metabolite B the calculated dietary burden is between dose group 2 (0.020 mg/kg bw) and dose group 3 (0.103 mg/kg bw). In animal matrices no residues above the LOQ of 0.1 mg/kg could be observed. Nevertheless, in milk residues above the limit of determination were found. Based on the calculated dietary burden metabolite B residues of 0.05 mg/kg (0.075 mg/kg, expressed as chloridazon equivalents) in milk might be possible.
Therefore, an MRL of 0.1 mg/kg for all animal products is proposed.

B.7.13 Proposed EU Import tolerances and justification for the acceptability of those residues

Import tolerances are not considered necessary.

B.7.14 Basis for differences, if any, in conclusion reached having regard to established or proposed Codex MRLs

There are no codex MRLs set for chloridazon.

B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)

B.7.15.1 Theoretical Maximum Daily Intake (TMDI)

The dietary risk assessment on national basis (NTMDI, NEDI, German model) and international level (TMDI, IEDI, WHO European diet) is based on an ADI value of 0.1 mg/kg bw for chloridazon (see B.6.10). Where an MRL could not be recommended the LOQ of 0.13 mg/kg is used in the NTMDI and TMDI calculation. It can be concluded that the long-term intake of residues of chloridazon resulting from uses that have been considered is unlikely to present a public health concern.

Table B.7.15-1: NTMDI-Calculation for chloridazon (German model)

Active substance:	Chloridazon				
ADI (mg/kg bw):	0.1000				
Mean food consumption (g/d) of a 4 to 6 years old girl					
Food	**raw[1]**	**processed[2]**	**whole**	**MRL (mg/kg)**	**Intake (mg/kg) bw**
1. FRUITS AND TREE NUTS	72.0	89.3	161.3	0.13	0.00155326
2. VEGETABLES	33.0	75.4	108.4		0.00000000
(i) Root and tuber vegetables	3.3	11.1	14.4	0.50	0.00053333
(ii) Bulb vegetables	2.9	8.0	10.9	0.20	0.00016148
(iii) Fruiting vegetables	18.9	13.1	32.0	0.13	0.00030815
(iv) Brassica vegetables	2.5	22.7	25.2	0.13	0.00024267
(v) Leaf vegetables and fresh herbs	4.8	3.6	8.4	0.13	0.00008089
Beet leaves (chard)		0.2	0.2	3.0	0.00004444
(vi) Legume vegetables (fresh)	0.1	7.8	7.9	0.13	0.00007607
(vii) Stem vegetables	0.3	6.2	6.5	0.13	0.00006259
(viii) Fungi	0.2	2.8	3.0	0.13	0.00002889
3. PULSES		1.5	1.5	0.13	0.00001444
4. OIL SEEDS	0.3	11.0	11.3	0.13	0.00010881
5. POTATOES		71.1	71.1	0.13	0.00068467
6. TEA*					0.00000000
7. HOPS*					0.00000000
8. CEREALS	0.2	107.8	108.0	0.13	0.00104000
9. SPICES (without ginger)	0.1	0.1	0.2	0.13	0.00000193
10. GINGER		0.2	0.2	0.13	0.00000193
11. TEA LIKE PRODUCTS		0.3	0.3	0.13	0.00000289
12. COCOA BEANS		29.4	29.4	0.13	0.00028311
13. SUGAR BEET		0.3	0.3	0.30	0.00000667
Intake whole (mg/kg bw):	0.0052				
Percent of ADI (%):	5.25				
Explanations:					
1. raw = without any preparation/processing					
2. processed = e.g. washed, peeled, cooked, baked, preserves					
* Food which is normally not consumed by a 4 to 6 years old girl					
Mean food consumption (g/d) of a 36 to 50 years old woman					
Food	**raw[1]**	**processed[2]**	**whole**	**MRL (mg/kg)**	**Intake (mg/kg) bw**
Wine grapes (wine)		97.6	97.6	0.13	0.00021147
Tea		1.1	1.1	0.13	0.00000238
Hops		4.9	4.9	0.13	0.00001062
Coffee beans (raw)		26.5	26.5	0.13	0.00005742
Intake whole (mg/kg bw):	0.0003				
Percent of ADI (%):	0.28				

Table B.7.15-2: NEDI-Calculation for chloridazon (German model)

Active substance:	Chloridazon				
ADI (mg/kg bw):	0.1000				
Mean food consumption (g/d) of a 4 to 6 years old girl					
Food	**raw[1]**	**processed[2]**	**whole**	**MRL (mg/kg)**	**Intake (mg/kg) bw**
Root and tuber vegetables	3.3	11.1	14.4	0.50	0.00053333
Bulb vegetables	2.9	8.0	10.9	0.20	0.00016148
Beet leaves (chard)		0.2	0.2	3.0	0.00004444
Sugar beet		0.3	0.3	0.30	0.00000667
Intake whole (mg/kg bw):	0.0007				
Percent of ADI (%):	0.47				
Explanations:					
1. raw = without any preparation/processing					
2. processed = e.g. washed, peeled, cooked, baked, preserves					
* Food which is normally not consumed by a 4 to 6 years old girl					

Table B.7.15-3: TMDI-Calculation European diet (WHO, 1998)

Active substance:	Chloridazon		
ADI (mg/kg bw):	0.1000		
Mean food consumption in g/d (WHO European diet (1998))			
Food	**Consumption (g/day)**	**MRL (mg/kg)**	**Intake (mg/kg bw)**
FOOD OF PLANT ORIGIN	*1253.4*		
1. FRUITS AND TREE NUTS	*287.8*	0.13	0.00062357
2. VEGETABLES	*339.0*		
(i) Root and tuber vegetables	*36.3*		
Beetroot	2.0	0.50	0.00001667
Carrots	22.0	0.13	0.00004767
Celeriac[4]	2.0	0.13	0.00000433
Horseradish		0.13	0.00000000
Jerusalem artichokes		0.13	0.00000000
Parsnips	2.0	0.13	0.00000433
Parsley root		0.13	0.00000000
Radishes	2.0	0.13	0.00000433
Salsify		0.13	0.00000000
Sweet potatoes	1.3	0.13	0.00000282
Swedes	2.0	0.13	0.00000433
Turnips	2.0	0.13	0.00000433
Yams		0.13	0.00000000
Chicory roots	1.0	0.13	0.00000217
(ii) Bulb vegetables	*31.8*		
Garlic	3.0	0.13	0.00000650
Onions (including shallots)	27.8	0.20	0.00009267
Spring onions	1.0	0.13	0.00000217
(iii) Fruiting vegetables	*129.1*	0.13	0.00027972
(iv) Brassica vegetables	*47.4*	0.13	0.00010270

Active substance:	Chloridazon		
ADI (mg/kg bw):	0.1000		
Mean food consumption in g/d (WHO European diet (1998))			
Food	**Consumption (g/day)**	**MRL (mg/kg)**	**Intake (mg/kg bw)**
(v) Leaf vegetables and fresh herbs	*51.3*	0.13	0.00011115
Beet leaves (chard)	*0.1*	3.0	0.00000500
(vi) Legume vegetables (fresh)	*26.0*	0.13	0.00005633
(vii) Stem vegetables	*13.1*	0.13	0.00002838
(viii) Fungi	*4.0*	0.13	0.00000867
3. PULSES	*9.4*	0.13	0.00002037
4. OIL SEEDS	*28.3*	0.13	0.00006132
5. POTATOES	240.8	0.13	0.00052173
6. TEA	2.3	0.13	0.00000498
7. HOPS[2]	4.9	0.13	0.00001062
8. CEREALS	*223.3*	0.13	0.00048382
9. SPICES (without ginger)	0.4	0.13	0.00000087
10. GINGER	0.1	0.13	0.00000022
11. TEA LIKE PRODUCTS			
12. COCOA BEANS	3.1	0.13	0.00000672
13. SUGAR BEET	106.1	0.30	0.00053050
14. COFFEE BEANS	7.9	0.13	0.00001712
FOOD OF ANIMAL ORIGIN	*610.0*	0.10	0.00101667
Intake whole (mg/kg bw):	0.00426633		
Percent of ADI (%):	4.27		
Explanations:			
1. strawberries, cane fruit and other small fruit and berries without wild fruit and wild berries			
2. value from German food consumption			
3. 31st session of the CCPR			
4. value from 1994 table			

Table B.7.15-4: IEDI-Calculation European diet (WHO, 1998)

Active substance:	Chloridazon		
ADI (mg/kg bw):	0.1		
Mean food consumption in g/d (WHO European diet (1998))			
Food	**Consumption (g/day)**	**MRL (mg/kg)**	**Intake (mg/kg bw)**
Root and tuber vegetables	36.3	0.50	0.00030250
Bulb vegetables	31.8	0.20	0.00010600
Beet leaves (chard)	0.1	3.0	0.00000500
Sugar beet	106.1	0.30	0.00053050
Intake whole (mg/kg bw):	0.000944		
Percent of ADI (%):	0.94		
Explanations:			
1. strawberries, cane fruit and other small fruit and berries without wild fruit and wild berries			
2. value from German food consumption			
3. 31st session of the CCPR			
4. value from 1994 table			

B.7.15.2 Estimated Maximum Daily Intake (EMDI)

Chloridazon does not represent an acute risk to consumers. Therefore, an acute reference dose is not considered necessary.

B.7.16 Summary and evaluation of residue behaviour (Annex IIA 6.10; Annex IIIA 8.9)

B.7.16.1 Metabolism in plants

The metabolism on plants was investigated with the ^{14}C-labelled active substance in sugar beets. Chloridazon was used was in pre- and post-emergence application with a rate 2 kg as/ha up to 5 kg as/ha.
In the pre-emergence application the active substance was found only in minor parts to the total amount of residues. In the first trial metabolite A (glucose-chloridazon) and in the second one the soil metabolite B formed the main residue with an amount of 60 % each.
The post-emergence application showed only little transformation of the active substance. The amount of unchanged chloridazon in both trials counted over 75 % of the total residue.

Two metabolic pathways for chloridazon in plants are proposed:
glucosidation of chloridazon to glucose-chloridazon (metabolite A),
glucosidation of the soil metabolite B.

B.7.16.2 Metabolism in livestock

The metabolism in livestock animals was investigated in lactating goats and laying hens. The metabolic pathway is comparable in both species. The following biotransformation were observed:
dechlorination of chloridazon,
hydroxylation of the phenyl ring in para-position,
conjugation of para-hydroxy-chloridazon with sulfat.

B.7.16.3 Definition of the residue

plant material
Metabolism studies in plants were only performed on sugar beets. Therefore, a general residue definition for all plant materials is not possible.
Based on the submitted study, the following residue definition on root and tuber vegetables is proposed:

Residue for monitoring:	Chloridazon and metabolite B, expressed as chloridazon equivalents
Residue for risk assessment:	Chloridazon and its metabolites A and B, expressed as chloridazon equivalents

animal products

Residue for monitoring: Metabolite B, expressed as chloridazon

Residue for risk assessment: Sum of chloridazon and metabolite B, expressed as chloridazon equivalents

B.7.16.4 Residues in plants

In total 24 trial on sugar beets, 6 trials on red beets and 8 trials on onions were performed with an rate of 2.6 kg as/ha as pre-emergence application. At harvest the following residues could be found:

Residues in sugar beet roots after 98 to 183 days:
<0.13(13), 0.13(7), 0.14, 0.16, 0.18, 0.20 mg/kg chloridazon/metabolite A + metabolite B, calculated as chloridazon

STMR: 0.13 mg/kg
HR: 0.20 mg/kg

Residues in red beet roots after 93 days:
<0.13, 0.14, 0.16(3), 0.19 mg/kg chloridazon/metabolite A + metabolite B, calculated as chloridazon

STMR: 0.16 mg/kg
HR: 0.19 mg/kg

Residues in onion bulbs after 78 to 122 days:
<0.13(3), 0.14 mg/kg chloridazon/metabolite A + metabolite B, calculated as chloridazon
STMR: 0.13 mg/kg
HR: 0.14 mg/kg

Residues in onion bulbs after 108 to 146 days:
<0.13(4) mg/kg chloridazon + metabolite B, calculated as chloridazon
STMR: 0.13 mg/kg
HR: 0.13 mg/kg

Residues in red beet tops after 93 days:
0.62, 0.76, 0.86, 1.2, 1.4, 1.5 mg/kg chloridazon/metabolite A + metabolite B, calculated as chloridazon

STMR: 1.03 mg/kg HR: 1.5 mg/kg

B.7.16.5 Freezer storage stability

The freezer storage stability at < -5 °C of chloridazon, glucose-chloridazon (metabolite A) and the soil metabolite B was investigated over a period of at least 24 months.
In the matrices sugar beet root, sugar beet top, refined sugar, molasses and dried pulp no significant instability of residues (< 70 %) was observed.

B.7.16.6 Processing studies

The effect of degradation during industrial processing was investigated for chloridazon and the soil metabolite B. Test solutions of both substances were sealed and treated under conditions, that simulate the processes of pasteurisation, baking and cooking. Any decrease or transformation of the residues could no be observed.

The effect on the residue level by industrial could not be quantified, because the residue level in the raw agricultural commodity were below the LOQ.

B.7.16.7 Rotational crop studies

The studies on rotational crops indicate, that residues above the LOQ may occur in any succeeding crops. The trials performed with an application rate of 1.5x of the critical GAP showed residue levels in wheat grain above 1.0 mg/kg. In chard, radish, oat, wheat and sorghum no total ^{14}C-residues below 0.4 mg/kg (chloridazon equivalents) were found.

B.7.16.8 MRL proposal

Plant matrices:

Sugar beet:	0.3 mg/kg chloridazon and metabolite B, expressed as chloridazon equivalents
Red beet:	0.5 mg/kg chloridazon and metabolite B, expressed as chloridazon equivalents
Onion:	0.2 mg/kg chloridazon and metabolite B, expressed as chloridazon equivalents
Chard:	3 mg/kg chloridazon and metabolite B, expressed as chloridazon equivalents

Animal matrices:

All animal products:	0.1 mg/kg metabolite B, expressed as chloridazon

B.7.16.9 TMDI calculation

Long-term intake:
Based on the proposed MRLs and the LOQ of 0.13 mg/kg the TMDI calculation represents 5.3 % of the ADI for the German 4-6 years old girl and 4.3 % of the ADI for the adult of 60 kg body weight (WHO, 1998).
It can be concluded that the long-term intake of residues of chloridazon resulting from uses that have been considered is unlikely to present a public health concern.

Short-term intake:
Chloridazon does not represent an acute risk to consumers. Therefore, an acute reference dose is not considered necessary.

B.7.17 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-6.0; AIIA-6.1	Mayer, F.	1991	The metabolism of ^{14}C-chloridazon in sugar beets. 91/10553 GLP, unpublished RIP2004-851 RIP2002-2518	N	BAS
AIIA-6.0	Riley, M.E. and Movassaghi, S.	1996	Freezer storage stability of chloridazon (pyrazon) and metabolites in sugar beet root, tops, dried pulp, refined sugar and molasses. 96/5065 GLP, unpublished RIP2002-526	Y	BAS
AIIA-6.1	Hofmann, M.	1990	Plant uptake study with ^{14}C-chloridazon in sugar beets (pre-emergence). 90/0188 GLP, unpublished RIP2002-530	N	BAS
AIIA-6.1	Hofmann, M.	1990	Plant uptake study with ^{14}C-chloridazon in sugar beets (late application). 90/0033 GLP, unpublished RIP2002-529	N	BAS
AIIA-6.1	Hofmann, M.	1990	Plant uptake study with ^{14}C-chloridazon in sugar beets (post-emergence). 90/0032 GLP, unpublished RIP2002-528	N	BAS
AIIA-6.1	Hofmann, M.	1990	Plant uptake study with ^{14}C-chloridazon in sugar beets (pre-emergence). 90/0031 GLP, unpublished RIP2002-527	N	BAS
AIIA-6.2	Kampke-Thiel, K.	1999	The metabolism of ^{14}C-metabolite B (Reg. No. 014456) of chloridazon in laying hens. 99/11081 GLP, unpublished RIP2002-534	Y	BAS
AIIA-6.2	Kampke-Thiel, K.	1999	The metabolism of ^{14}C-metabolite B (Reg. No. 014456) of chloridazon in lactating goat. 99/11080 GLP, unpublished RIP2002-532	Y	BAS

[1] Only notifier listed

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AIIA-6.2	Kampke-Thiel, K. and Hartl, M.	2002	Amendment No. 1: The metabolism of ^{14}C-metabolite B (Reg. No. 014 456) of chloridazon in laying hens. 2002/1008532 GLP, unpublished RIP2003-915	Y	BAS
AIIA-6.2	Leibold, E. and Hoffmann, H.D.	1999	^{14}C-metabolite B of chloridazon - Absorption, distribution and excretion after repeated oral administration in laying hens. 1999/11788 GLP, unpublished RIP2003-906	Y	BAS
AIIA-6.2	Leibold, E. and Hoffmann, H.D.	1999	^{14}C-metabolite B of chloridazon - Absorption distribution and excretion after repeated oral administration in lactating goats. 1999/11787 GLP, unpublished RIP2003-905	Y	BAS
AIIA-6.2	Winkler, V. and Suter, P.	1992	Nature of the residue of ^{14}C-BAS 119 H in the lactating goat. 92/5162 GLP, unpublished RIP2002-531	N	BAS
AIIA-6.2	Wu, D.	1991	Nature of the residue of ^{14}C-BAS 119 H in the laying hen. 91/5190 GLP, unpublished RIP2002-533	N	BAS
AIIA-6.2	Zehr, R.D.	1996	Accountability of BASF method no. D9407 in goat milk. 1996/5109 GLP, unpublished RIP2003-914	Y	BAS
AIIA-6.3	Balluff, M.	2001	Field residue study for the determination of residues of the active ingredient(s) after the maximum number of applications under open field conditions with BAS 119 33 H in sugar beet in Northern Europe, 2000. 2001/1009078 GLP, unpublished RIP2003-916	Y	BAS

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AIIA-6.3	Freschi, G.	2002	Determination of residues of chloridazon, metabolite A and metabolite B in sugar beet samples (leaves+top, roots and shoots) after an application of Chloridazon 65 % WG in two Italian trials, 2001. 2002/1011936 GLP, unpublished RIP2003-917	Y	BAS
AIIA-6.3	Freschi, G.	2002	Determination of residues of chloridazon, metabolite A and metabolite B in sugar beet samples after an application of Chloridazon 65 % WG in two Spanish trials. 2002/1011937 GLP, unpublished RIP2003-919	Y	BAS
AIIA-6.3	Freschi, G.	2002	Determination of residues of chloridazon, metabolite A and metabolite B in sugar beet samples after an application of Chloridazon 65 % WG in two Italian trials. 2002/1011934 GLP, unpublished RIP2003-913	Y	BAS
AIIA-6.3	Freschi, G.	2002	Determination of residues of chloridazon, metabolite A and metabolite B in sugar beet samples (leaves+top, roots and shoots) after an application of Chloridazon 65 % WG in two Spanish trials, 2001. 2002/1011935 GLP, unpublished RIP2003-908	Y	BAS
AIIA-6.3	Jones, S.	2003	Final report amendment: Study on the residue behaviour of BAS 119 H in onion after application of BAS 119 33 H under field conditions in Belgium, Denmark, Sweden 2001. 2003/1009456 GLP, unpublished RIP2003-921	Y	BAS
AIIA-6.3	Jones, S.	2003	Study on the residue behaviour of BAS 119 H in onion after application of BAS 119 33 H under field conditions in Belgium, Denmark, Sweden 2001. 2003/1000944 GLP, unpublished RIP2003-920	Y	BAS

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AIIA-6.3	Keller, E.	2000	Summary of residue trials with chloridazon formulations in red beets in 1978. 2000/1013265 not GLP, unpublished RIP2002-536	Y	BAS
AIIA-6.3	Keller, E.	2000	Summary of residue trials with chloridazon formulations in sugar beets in 1975, 1976 and 1977. 2000/1013266 not GLP, unpublished RIP2002-535	Y	BAS
AIIA-6.3	Raunft, E. et al.	2003	Study on the residue behaviour of chloridazon in onions after treatment with BAS 119 33 H under field conditions in Germany and Great Britain, 2000. 2003/1000943 GLP, unpublished RIP2003-1007 RIP2003-881	Y	BAS
AIIA-6.4	Copeland, G.L. and Stanovick, R.P.	1970	Development of analytical methods and analysis for residues in samples of whole milk, muscle, tissue, kidney tissue, liver tissue and fat tissue from cows. 1970/10014 not GLP, unpublished RIP2003-922	N	BAS
AIIA-6.4	Weatherholtz, W.M.	1970	28-day feeding and residue study - dairy cows "dephenylated" pyrazon, pyrazon-N-glucoside Final Report. 70/5003 not GLP, unpublished RIP2002-538	N	BAS
AIIA-6.4	Zehr, R.D. and Riley, M.E.	1996	A meat and milk magnitude of the residue study with BAS 119 H (chloridazon) in lactating dairy cows. 96/5063 GLP, unpublished RIP2002-537	Y	BAS
AIIA-6.5.1	von Götz, N.	2001	Hydrolysis of BAS 119 H and metabolite B of chloridazon at 90 °C, 100 °C, and 120 °C. 2001/1008958 GLP, unpublished RIP2003-909	Y	BAS

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AIIA-6.5.2	Schulz, H.	2001	Determination of the residues of chloridazon in sugar beet and processed products following treatment with BAS 119 33 H under field conditions in Germany 2000. 2001/1009077 GLP, unpublished RIP2003-910	Y	BAS
AIIA-6.6	Ellenson, J.L.	1993	Fate of 14C-BAS 119 H in emergency, fall and annual replant confined rotational crops. 93/5001 GLP, unpublished RIP2002-539	Y	BAS
AIIA-6.6	Zehr, R.D.	1996	Pyramin herbicide limited field rotational crop study. 96/5179 GLP, unpublished RIP2002-540	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft