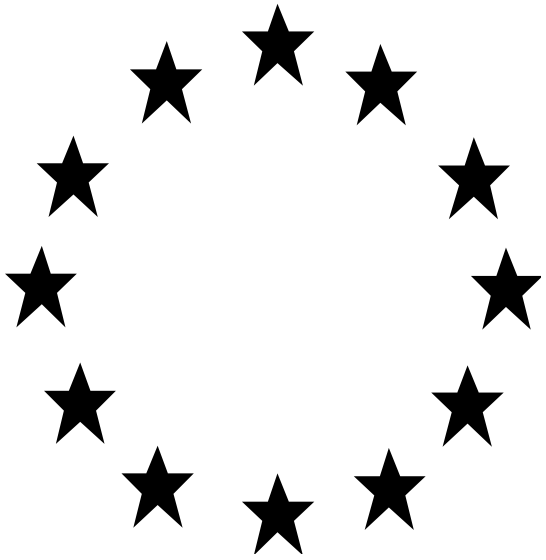


Draft Assessment Report (DAR)

- 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.6, part 1

July 2005

Annex B

Chloridazon

B-6: Toxicology and metabolism

B.6 Toxicology and metabolism

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

After single oral administration of chloridazon to male and female rats at dose levels of 20 mg/kg and 200 mg/kg bw, the active ingredient is rapidly absorbed from the gastro-intestinal tract. The absorbed material is rapidly excreted mostly via urine (between 85 % and 90 %) and feces (7 % - 26 %) with a half-life of 16 to 49 hours. There were no significant differences between the high and low dose level or any sex-related differences regarding rates and routes of excretion. As a comparison of the excretion balance after oral vs. intravenous administration showed, virtually all radioactivity in the feces was related to biliary excretion, which was found to be 12 % to 37 % of the dose. The bioavailability was approximated to be 96-100 %.

Excretion after a 14-day pre-treatment was even faster than after a single low dose. Cmax increased less than proportionally with dose. The AUC was linear over the tested dose range for male rats, while in females the AUC value increased slightly less than proportionally with the dose indicating a marginally lower absorption at the high dose level.

After a 7-day treatment with 20 mg/kg bw, highest amounts of radioactivity were found in the heart, adrenal glands and in the gastro-intestinal tract. There was a steady decline in the radioactivity in all organs and tissues indicating that chloridazon has no accumulating potential.

After oral dosing, chloridazon was rapidly metabolised in the rat by oxidative mechanisms. The main transformation is the hydroxylation of the parent compound at the phenyl ring moiety. This metabolite is then converted either to its glucuronide or sulfate. Subsequent hydroxylation at the phenyl ring or - to a lesser extent - dechlorination of the sulfate conjugate was also observed. Three major of at least 9 metabolites were found in urine.

B.6.1.1 Absorption, distribution and excretion

Report: Hoffmann H.D., Hildebrand B., 1991, Study on the biokinetics of ^{14}C Reg. No. 13 033 in rats
[redacted]
unpublished
BASF RegDoc# 1991/10585

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

Guideline: OECD 417, EPA 85-1

Deviations: None

Acceptability: The study is considered to be acceptable.

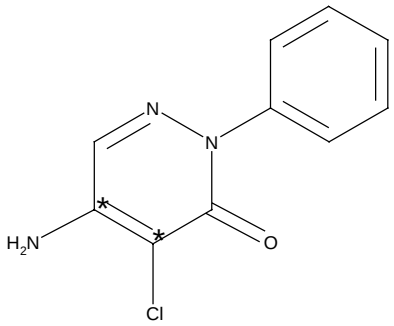
Material and Methods:

Test material: The study was performed using ^{14}C-chloridazon (Reg. No. 13 033), which was radiolabelled at two sites of the pyridazin ring moiety [see Figure B.6.1-1].

[pyridazin-4,5-14C]-chloridazon: Batch No.: 249/158/7, specific radioactivity: 15.87 MBq/mg (428.5 µCi/mg), radiochemical purity: > 99 %

Unlabelled chloridazon: Batch No: 687 121, Purity: 99.8 %

Figure B.6.1-1: Structure of ^{14}C-labelled chloridazon:



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

Test animals: Wistar rats
Stock solutions of labelled and unlabelled material were prepared in acetonitrile. Appropriate proportions were mixed to achieve the required specific activity. The solvent was then evaporated and the remaining material was suspended in 0.5 % aqueous tylose. Prior to dosing the samples were sonicated to achieve a homogenous suspension. For intravenous administration the material was prepared as an aqueous mixture of saline and Pluriol (80:20, *v/v*).
The stability of the test item in 0.5 % aqueous tylose or water (saline) has been shown analytically.
The experiments that were carried out are summarised in the following tables:

Table B.6.1-1: Summary of dose groups and analysed samples

Experiment No.* Dose Groups**	**1 A**	**2 B**	**3 C**	**4 D**	**10 E**	**11**
Purpose	excretion balance, metabolite patterns	excretion balance, metabolite patterns	excretion balance, metabolite patterns	excretion balance, metabolite patterns	biliary excretion, metabolite identification	biliary excretion
Dosing	oral high	oral low	i.v. low	Multiple oral low***	oral high	oral low
Nominal dose level (mg/kg bw)	200	20	10	20	200	20
No. of animals (male/female)	5/5	5/5	5/5	5/5	3/3	3/3
Duration [h]	72	72	72	96	48	48
Samples	urine, feces, tissues, exhaled air	urine, feces, tissues	urine, feces, tissues	urine, feces, tissues	bile	bile
Methods of analysis	total ^{14}C	total ^{14}C	total ^{14}C	total ^{14}C	total ^{14}C	total ^{14}C

* Designation used in the biokinetics part [see 1991/10585 Hoffmann H.D., Hildebrand B., 1991]
** Designation used in the metabolism part [see 1991/10524 Bornemann V., 1991]
*** 4 non-radiolabelled doses followed by one ^{14}C-labelled dose

Table B.6.1-2: Summary of dose groups and analysed samples (continued)

Experiment No.*	**5**	**6**	**7**	**8**	**9**
Purpose	blood/plasma level	blood/plasma level	blood/plasma level	tissue distribu-tion	tissue distribu-tion
Dosing	oral high	oral low	oral high	oral high	Multiple oral low**
Nominal dose level (mg/kg bw)	200	20	200	200	20
No. of animals (male/female)	5/5	5/5	5/5	6/6 (1 per time point)	10/10 (2 per time point)
Duration [h]	72	72	8	72	72
Samples	blood/plasma	blood/plasma	blood/plasma	tissues	tissues
Methods of analysis	total ^{14}C	total ^{14}C	TLC analysis for parent compound	whole body autoradiography	total ^{14}C tissue analysis

* Designation used in the biokinetics part [see 1991/10585 Hoffmann H.D., Hildebrand B., 1991]

** 7 daily doses

For the excretion balance experiments (No. 1-4), animals were placed in metabolism cages after dosing and excreta were collected in time intervals of 24 hours up to 168 hours or until 95 % of the applied radioactivity was excreted. In the single oral high dose the exhaled air was collected. At the end of the collection period the following organs and tissues were prepared for analysis of residual radioactivity: heart, lungs, liver, spleen, kidneys, adrenal glands, gonads, muscle, brain, skin, fat tissue, bone, thyroid glands, pancreas, stomach and stomach contents, gut and gut contents, blood plasma and the carcass.
In the bile excretion experiments (No. 10 and 11), animals were anaesthetised and bile was collected via a catheter in 3-hour time intervals for up to 48 hours after dosing.
For the blood/plasma pharmacokinetics experiments No. 5 and 6, animals were placed in metabolism cages after treatment and blood samples were drawn at the following time points after dosing: 0.5, 1, 2, 4, 8, 24, 48, 72 hours. The blood and plasma samples were checked for total radioactivity. In the blood/plasma level experiment No. 7, blood was sampled at 4 time points and the resulting plasma samples were analysed for the content of parent compound by TLC.
In the tissue distribution experiment No. 8, one animal was sacrificed at each of the following time points: 1, 2, 4, 8, 24 and 72 hours after dosing, and subjected to whole body autoradiog-raphy. In the tissue distribution experiment No. 9, two animals of each sex were sacrificed at the following time points after the last of seven daily doses: 1, 4, 8, 24 and 72 hours. Radioactivity was determined in the same organs/tissues as for the excretion balance experiments (see enumeration above).

Findings:

Excretion balance

The excretion of chloridazon is summarised in the following tables:

Table B.6.1-3: Excretion balance (in percent of administered radioactivity) after administration of ^{14}C-chloridazon to male and female rats

Dose		20 mg/kg bw p.o.		200 mg/kg bw p.o.		10 mg/kg bw i.v.		20 mg/kg bw multiple p.o.*	
Sex		Male	Female	Male	Female	Male	Female	Male	Female
Urine	0 – 12 h	n.d.	n.d.	n.d.	n.d.	82.80	75.80	n.d.	n.d.
	12/0 - 24 h	86.63	84.75	66.05	57.25	4.86	10.84	83.02	55.26
	24 - 48 h	2.58	3.72	15.07	26.27	1.17	2.34	2.62	3.01
	48 - 72 h	0.27	0.52	3.71	4.22	0.37	0.47	0.79	0.65
	72 - 96 h	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.29	0.48
Subtotal Urine		89.48	88.99	84.83	87.75	89.20	89.45	86.72	59.40
Feces	0 – 12 h	n.d.	n.d.	n.d.	n.d.	8.72	6.22	n.d.	n.d.
	12/0 - 24 h	11.97	15.53	1.66	1.18	3.49	17.24	16.52	19.65
	24 - 48 h	0.93	1.49	3.84	7.67	2.00	2.03	2.89	2.01
	48 - 72 h	0.24	0.24	1.38	1.90	0.37	0.29	0.59	0.18
	72 - 96 h	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.23	0.04
Subtotal Feces		13.14	17.26	6.88	10.75	14.58	25.78	20.23	21.88
Cagewash		2.16	1.25	2.29	1.76	2.05	1.91	2.53	2.42
Tissues/Carcass		0.60	0.36	3.29	3.04	0.51	1.10	0.28	0.25
Total		105.38	107.86	97.29	103.29	106.34	118.24	109.76	83.95

* 14 non-radiolabelled doses followed by one ^{14}C-labelled dose
n.d. not determined

Table B.6.1-4: Excretion via the bile (in percent of administered radioactivity) after administration of ^{14}C-chloridazon to male and female rats

Dose	20 mg/kg bw p.o.		200 mg/kg bw p.o.	
Sex	Male	Female	Male	Female
0 - 6 h	27.30	8.85	3.72	6.24
6 - 12 h	8.25	2.01	2.86	5.66
12 - 24 h	1.15	0.56	4.39	9.02
24 - 36 h	0.56	0.26	3.29	4.72
36 - 48 h	0.13	0.13	0.81	0.84
0 - 48 h	37.39	11.81	15.07	26.48

In all dose groups and experiments, the total amount of radioactivity was almost completely excreted, predominantly via the urine: 72-96 hours after administration 59 % to 90 % of the dose were recovered from urine and 7 % to 26 % from feces. No radioactivity was detected in the exhaled air. Already within the first 24 hours after dosing, 55 % - 88 % of the administered radioactivity was found in urine and 1 % - 23 % in feces. Radioactivity remaining in tissues and organs 72 hours or 96 hours post dosing was between 0.3 % and 3.3 % of the dose. The overall recovery of radioactivity was in the range of 84 % to 118 %. No sex-related differences regarding the route and time course of excretion were apparent.
Comparing the results from the single oral high and low dose excretion balance, it can be concluded that there is no apparent difference in the kinetics of chloridazon at 20 mg/kg or 200 mg/kg bw. The excretion balance after intravenous application is very similar to the one after oral dosing, suggesting that the radioactivity in feces results from biliary excretion. This conclusion is confirmed by the experimental data, which found biliary excretion accounting for 12 % - 27 % of the administered dose in females and 15 % - 37 % of the administered dose in males [see Table B.6.1-4]. Since the amount of radioactivity excreted via bile and urine essentially reflects the absorbed proportion of the dose, the extrapolated total excretion

and hence the bioavailability is assumed to be in the range of 96 % to 100 %, similarly for both dose levels, based on the amount of radioactivity excreted from 0 - 48 hours.

In the multiple low dose experiment an unusually low recovery was found for females. The radioactivity found in the feces, however, was comparable to that of the males and to the single oral low dose groups. Therefore, the low amount of radioactivity found in the urine accounted for the low total recovery. As there was no apparent sex difference in the single low dose experiment the low recovery is considered as an artifact due to the conduct of the study.

A comparison of the excretion balance between single and multiple oral low dose administration shows that the elimination of chloridazon after multiple oral low doses is as efficient as after a single oral low dose. In both cases elimination is virtually complete within 24 hours, indicating that chloridazon has no potential for accumulation.

Kinetic parameters

The key biokinetic parameters are summarised in Table B.6.1-5. After oral administration at both dose levels the peak plasma level was reached within 0.9 hours post dose. The plasma level maxima ranged between 11.3 µg eq/g and 13.6 µg eq/g after dosing of 20 mg/kg bw and from 61.0 µg eq/g to 73.9 µg eq/g after dosing of 200 mg/kg bw. These results indicate that a 10-fold higher dose leads to a less than proportional increase of maximum plasma levels (Cmax) within comparable time though (Tmax).
Concentrations of radioactivity in blood were lower than in plasma, indicating that major parts of the radioactivity are in plasma and not bound to erythrocytes or other blood cells.
After reaching the peak plasma level (Cmax), radioactivity declined in a biphasic manner with terminal half lives between 16 hours and 50 hours.

The AUC was approximately linear over the dose range in male rats, while in females the plasma AUC-value increased slightly less than proportionally with the dose indicating a marginally lower absorption at the high dose level.

Table B.6.1-5: Biokinetic parameters derived from plasma level vs. time curves after single oral administration of ^{14}C-chloridazon to male and female rats

Dose level	**20 mg/kg bw p.o.**		**200 mg/kg bw p.o.**	
Sex	Male	Female	Male	Female
Cmax [µg/g]	11.27	13.62	73.94	61.00
T_{max} [h]	0.6	0.7	0.9	0.9
t1/2 [h]	29.80	35.35	15.58	48.96
AUC [µg*h/g]	45.93	85.41	467.35	577.82

Tissue distribution

One hour after the last of seven consecutive doses of 20 mg/kg bw, highest amounts of radioactivity were found in the adrenal gland (males: 100 µg eq/g wet tissue; females: 40 µg eq/g), heart (males: 95 µg/g, females: 108 µg/g) and in the gastro-intestinal tract (males: 74 µg/g and 51 µg/g; females: 31 µg/g and 67 µg/g in the contents of stomach and gut, respectively). Lowest levels were found in the thyroid, brain, fat, kidney and bones (4 µg/g - 9 µg/g tissue) for both sexes. Blood and plasma contained approximately 12 µg/g and 19 µg/g, respectively.
At 72 hours after dosing the residual radioactivity had declined continuously in all tissues and dropped below 1 µg/g except for heart and adrenal glands, which showed concentrations of 1.4 µg/g and 1.1 µg/g in males and of 1.8 µg/g and 3.5 µg/g in females. Blood and plasma

contained 0.90 µg/g and 0.13 µg/g in males and 0.91 µg/g and 0.16 µg/g in females, respectively.
Whole body autoradiography confirmed the tissue analysis. There were no organs detected with remarkably high levels of radioactivity.

Conclusion:
After single oral administration of ^{14}C-chloridazon to male and female rats at dose levels of 20 mg/kg and 200 mg/kg bw, the active ingredient is rapidly absorbed from the gastro-intestinal tract. The absorbed material is rapidly excreted mostly via urine (between 85 % and 90 %) and feces (7 % - 26 %) with a half-life of 16 hours to 49 hours. There were no significant differences between the high and low dose level or any sex-related differences regarding rates and routes of excretion. As a comparison of the excretion balance after oral vs. intravenous administration showed, virtually all radioactivity in the feces was related to biliary excretion, which was found to be 12 % to 37 % of the dose. Excretion after a 14-day pre-treatment was even faster than after a single low dose. Cmax increased less than proportionally with dose. The AUC was linear over the tested dose range for male rats, while in females the AUC value increased slightly less than proportionally with the dose, indicating a marginally lower absorption at the high dose level.
The bioavailability was approximately 96 % to 100 %. After a 7-day treatment with 20 mg/kg bw, highest amounts of radioactivity were found in the heart, adrenal glands, and in the gastro-intestinal tract. There was a steady decline in the radioactivity in all organs and tissues indicating that chloridazon has no potential for accumulation.

B.6.1.2 Metabolism

Report: Bornemann V., 1991, The metabolism of ^{14}C-chloridazon (BAS 119 H) in the rat. The identification and quantification of metabolites [redacted]
unpublished
BASF RegDoc# 1991/10524

GLP: Yes (laboratory certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 3180, 6500 Mainz)

Guideline: EPA GUIDELINES, SUBDIV.F, 85-1, OECD 417

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: The study was performed using ^{14}C-chloridazon (Reg. No. 13 033), which was radiolabelled at two positions of the pyridazin ring moiety [see figure B.6.1-1]. For batch-specific data of the labelled and unlabelled test item, see chapter 6.1.1.

Test animals: Wistar rats
The metabolism of chloridazon was investigated using the excreta (urine, feces and bile) of the absorption, distribution, metabolism and excretion study [see 1991/10585, Hoffmann H.D., Hildebrand B., 1991]. The excreta were obtained from the following groups:

Excretion balance, oral high dose (200 mg/kg bw) - Dose Group A.
Excretion balance, oral low dose (20 mg/kg bw) - Dose Group B.
Excretion balance, intravenous dose (10 mg/kg bw) - Dose Group C.
Excretion balance, multiple oral low dose (20 mg/kg bw), 14 daily doses of unlabelled material followed by single oral dose of radioactive material (14+1) - Dose Group D.
Excretion via the bile, oral high dose (200 mg/kg bw) - Dose Group E.

For a more detailed description of the experimental part of the study please refer to chapter 6.1.1.

Kidneys, liver and other organs and tissues did not contain sufficient amounts of radioactivity to allow the isolation or identification of metabolites.

The metabolite patterns were established using reversed phase HPLC. For isolation, characterisation and identification of metabolites, semi-preparative HPLC, TLC, mass spectrometry and nuclear magnetic resonance spectroscopy were used. Urine samples were submitted to treatment with the following enzymes to determine conjugation: glucuronidase, arylsulfatase, hesperidinase, β-glucosidase and acid hydrolysis. Co-chromatography with reference standards was also used for the identification of metabolites.

Findings:

In general rather similar metabolite patterns were found regardless of dose, route and duration of administration or sex.

Analysis of the urine indicated that at least nine compounds were excreted via this route. The main urinary metabolites were identified to be the phenyl ring-hydroxylated chloridazon (BH 119-4-O) and glucuronide or sulfate conjugates thereof. Minor metabolites consisted of sulfate conjugates of di-hydroxylated chloridazon and dechlorinated hydroxy-chloridazon. Unchanged parent compound was detected only at very low levels (approximately 1 % to 4 % of dose).

Some of the urinary metabolites were also found in feces and the bile. The main metabolite in feces was again the phenyl ring-hydroxylated chloridazon (BH 119-4-OH) while the most predominant metabolites in bile were BH 119-4-OH and its glucuronide. Only minor amounts of unchanged parent compound were detected in feces.

All identified metabolites in urine and feces are summarised in Table B.6.1-6 and Table B.6.1-7. Their structures can be found in Table B.6.1-8.

The results of this study show that chloridazon was rapidly metabolised in the rat by oxidative mechanisms.

The metabolic pathway of chloridazon is shown in Figure B.6.1-2.

Table B.6.1-6: Summary of identified metabolites in urine after single and multiple low and single high dose administration of ^{14}C-chloridazon. Total excretion in % of dose

Metabolite Identity *	Dose Group A (200 mg/kg single oral)		Dose Group B (20 mg/kg single oral)		Dose Group C (10 mg/kg single i.v.)		Dose Group D (20 mg/kg multiple oral)	
	Male	Female	Male	Female	Male	Female	Male	Female
	0 - 72 h		0 - 72 h		0 - 72 h		0 - 96 h	
Glucuronide of BH 119-4-OH (R1)	13.13	14.32	12.10	25.82	10.73	25.97	12.58	11.89
Sulfate conjugate of the phenyl ring dihydroxylated chloridazon (R2)	6.18	7.98	5.40	9.52	4.49	5.81	6.35	4.87
Sulfate conjugate of dechlorinated BH 119-4-OH (R6)	n.d.	n.d.	n.d.	1.86	1.95	4.68	0.27	0.10
Sulfate conjugate of BH 119-4-OH (R3)	49.91	13.75	61.29	19.87	52.95	22.18	48.58	13.24
Isomer of sulfate conjugate of BH 119-4-OH (R3 iso)	1.31	1.37	0.67	0.05	1.18	0.96	0.80	0.04
BH 119-4-OH (R4; Reg. No. 71551)	9.01	31.50	7.38	28.28	8.51	21.35	10.04	22.92
Chloridazon (R5; Reg. No. 13033)	2.14	2.43	n.d.	n.d.	2.58	3.93	1.32	n.d.

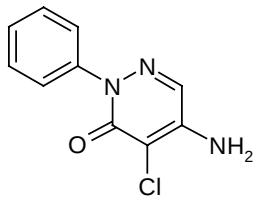
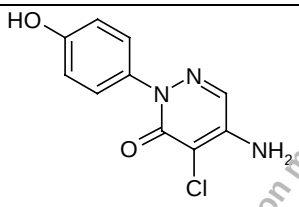
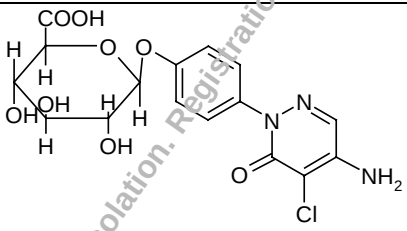
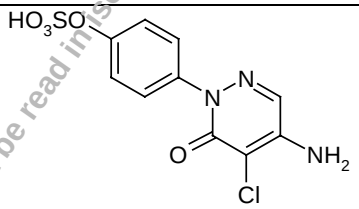
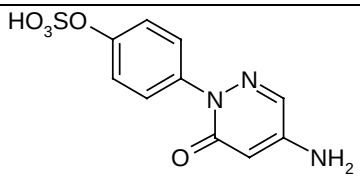
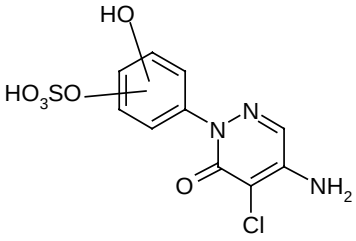
* The designations given in brackets are the metabolite codes as used in the report and the BASF internal registration number as far as available

Table B.6.1-7: Summary of identified metabolites in feces after single and multiple low and single high dose administration of ^{14}C-chloridazon. Total excretion in % of dose

Metabolite Identity *	Dose Group A (200 mg/kg single oral)		Dose Group B (20 mg/kg single oral)		Dose Group C (10 mg/kg single i.v.)		Dose Group D (20 mg/kg multiple oral)	
	Male	Female	Male	Female	Male	Female	Male	Female
	0 - 72 h		0 - 72 h		0 - 72 h		0 - 96 h	
BH 119-4-OH (FC2; Reg. No. 71551)	1.3	4.5	4.7	6.0	3.2	8.2	3.2	3.4
Chloridazon (FC4; Reg. No. 13033)	1.0	0.9	0.5	0.2	0.5	0.5	0.1	0.1

* The designations given in brackets are the metabolite codes as used in the report and the BASF internal registration number as far as available

Table B.6.1-8: Structures of identified metabolites in rat urine, feces, and bile

Metabolite Designation	Structure
Chloridazon (parent compound) (R5/FC4)	
BH 119-4-OH (Reg. No. 71551) (R4/FC2)	
Glucuronide of BH 119-4-OH (R1)	
Sulfate conjugate of BH 119-4-OH (R3)	
Sulfate conjugate of dechlorinated BH 119-4-OH (R6)	
Sulfate conjugate of the phenyl ring di-hydroxylated chloridazon (R2)	

Discussion:

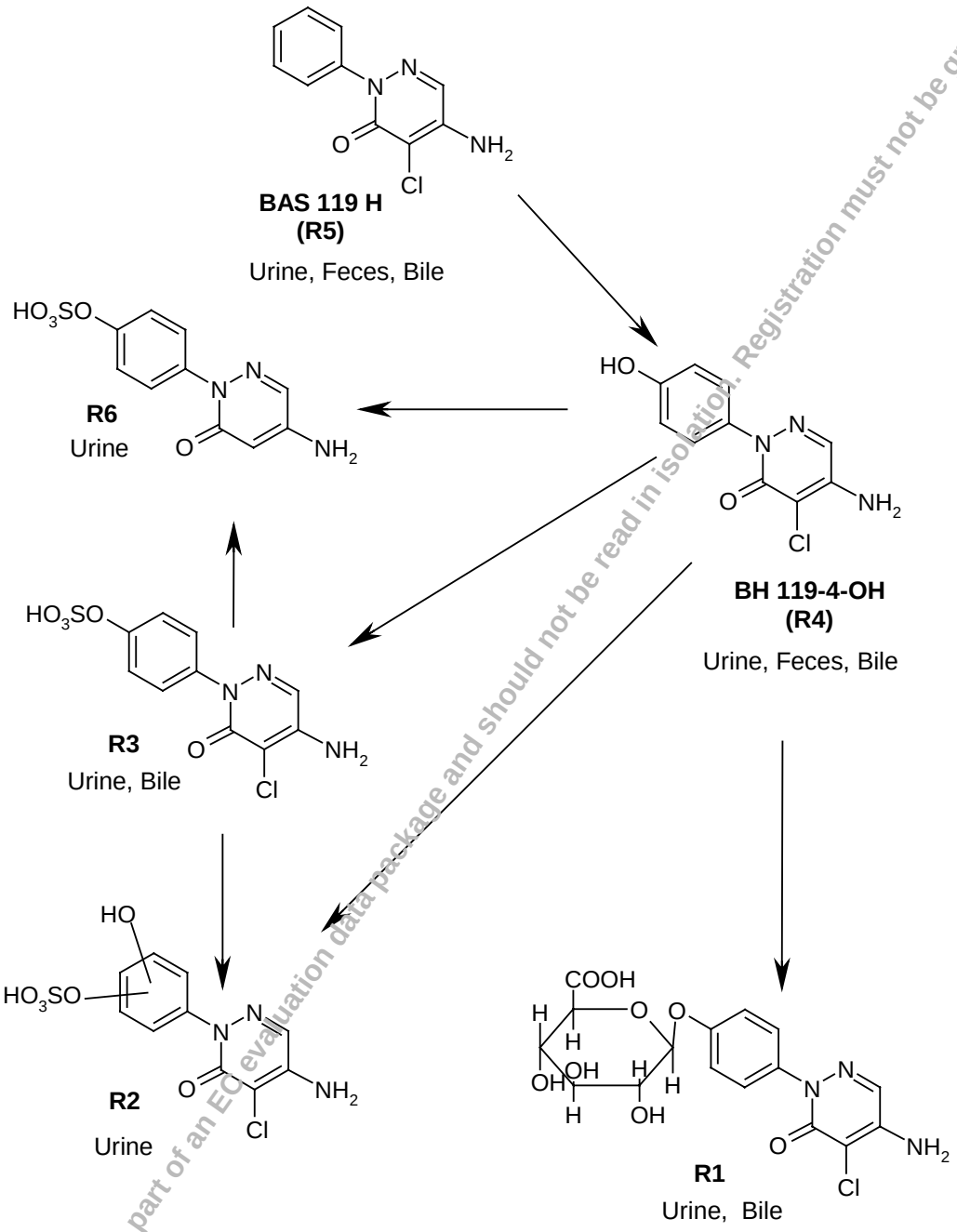
Qualitatively, the same metabolites were found in males and females. However, there were quantitative differences in metabolites. Higher amounts of sulphate metabolites were seen in urine of males and higher amounts of BH 119-4-OH in feces in females. This may be an expression of sexual differences in the conjugation and hydroxylation mechanisms. However, this effect is considered as toxicologically not relevant.

Conclusion:

After oral dosing chloridazon is rapidly metabolised in the rat. The metabolic transformations can be summarised as follows: Starting with the unchanged parent compound (only a few

percent being found in the excreta), the molecule is hydroxylated at the phenyl ring in para-position to the pyridazin moiety, rendering BH 119-4-OH. This metabolite is then converted either to its glucuronide or sulfate. Subsequent phenyl ring-hydroxylation or dechlorination (only to a minor extent) of the sulfate conjugate was also observed.

Figure B.6.1-2: Metabolic pathway of chloridazon in the rat:



B.6.1.3 Dermal absorption

Report:	Reference number III A 7.3/1 Leibold E., Ravenzwaay B., 2002 ^{14}C-BAS 119 H - Study of the dermal absorption in rats [redacted] BASF RegDoc# 2002/1008654
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Guideline:	OECD 427, EPA 870.7600
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: radiolabelled material: ^{14}C-chloridazon, Reg. No. 13 033, BAS 119 H, Batch/ purity: 43-72, > 95 %;
unlabelled material: BAS 119 50 H, Batch/purity: 98-1, 435.7 g/L (Reg. No. 13 033).
Test animals: Male Wistar rats

The absorption, distribution and excretion of radioactivity was studied in male Wistar rats following a single dermal administration of ^{14}C-BAS 119 H in the formulation (BAS 119 50 H) and 1/6 and 1/33 aqueous dilutions thereof. The low concentration was representative of the spray dilution to be used for field application. These preparations resulted in nominal dose levels of 4.3, 0.7 and 0.1 mg/cm² (corresponding nominally to about 43, 7 and 1.3 mg/animal and about 159, 26 and 4.5 mg/kg body weight). Groups of four animals were exposed according to the following regimen:

Table B.6.1-9: Experimental design

Duration of exposure [h]	1	4	10	24	10*	10
Sacrifice after [h]	1	4	10	24	24*	72

* only at low-dose level

Twenty-four hours prior to dosing the back shoulders of the rats were clipped free of hair and the area (about 10 cm²) was washed with acetone. A silicone ring was glued to the skin; the test substance preparation (about 10 µL/cm²) was administered with a syringe, which was weighed before and after application. A nylon mesh was then glued to the surface of the silicone ring and a porous bandage used to encircle the trunk of the animal.

The animals were dosed and then placed in metabolism cages in order to collect excreta up to 72 hours. After the respective exposure period, the protective cover was removed and the exposed skin was washed with a mild soap solution. For animals with a post-observation period, new gauze and a new bandage were applied and an additional skin wash was performed before sacrifice. At the end of the various collection periods animals were killed and the following specimens/tissues were checked for remaining radioactivity: excreta, blood cells, plasma, liver, kidneys, carcass, treated skin (application site) and non-treated skin areas (surrounding skin).

For balance estimates the cage wash and skin wash(es) as well as the protective cover (including the silicone ring) were also checked for radioactivity.

Findings:

The stability of the test item in the vehicle, the homogeneity and the actual concentrations of the test substance preparations were analytically verified.

Mean recoveries of radioactivity from all dose groups were in the range of 93.23 - 112.86 % of the total radioactivity administered. The largest proportion of radioactivity was recovered from skin wash. The relative amount of radioactivity absorbed (including excreta, cage wash, tissues/organs and carcass) generally increased with increasing exposure and sacrifice time and was in the same range for all dose levels. For the formulation concentrate (4.3 mg/cm^2), maximum relative absorption was about 0.42 %. For the aqueous dilutions, maximum relative absorption were 0.41 % and 0.67 % for the intermediate and low dose respectively. These results are summarised in the table below.

Table B.6.1-10: Mean percentage of radioactivity absorbed

Exposure time (hours)	Sacrifice time (hours)	4.3 mg/ cm²	0.43 mg/ cm²	0.1 mg/ cm²
		% absorbed	% absorbed	% absorbed
1	1	0.10	0.02	0.14
4	4	0.15	0.27	0.36
10	10	0.21	0.09	0.58
24	24	0.22	0.08	0.58
10	24	---	---	0.67
10	72	0.41	0.41	0.42

The radioactivity absorbed was excreted mainly via the urine. Highest tissue and organ concentrations of radioactivity were found in the remaining carcass. The concentrations in skin at the application site were 3.71 and 3.05 after 10 h application and 24 h and 72 h after sacrifice time respectively.

Conclusion:
The in vivo dermal absorption of BAS 119 H in rats was found to be very low under the study conditions chosen. The maximum relative absorption was approximately 0.42 % for the undiluted formulation and 0.67 % or less for the dilutions, depending on the duration of exposure and concentration.

Thus, for exposure assessment the following estimates for dermal absorption could be used: 0.7 % for the diluted product (spray application) and 0.4 % for the undiluted product (mixing/loading).

As the experiment was determined before serial non-detects were observed in excreta the amount located in the skin was considered as been absorbed and included into the calculation.

The dermal absorption was found to be approximately 4 % (10 h exposure including skin residues) both for the diluted and the undiluted product.

B.6.2 Acute toxicity including irritancy and skin sensitisation (Annex IIA 5.2)

Chloridazon is characterised by a low acute oral, dermal and inhalation toxicity in rats. Chloridazon is neither irritating to the skin nor to the eyes. Chloridazon is not a skin sensitiser in the Guinea pig Maximisation Test. To all relevant endpoints of this chapter isomer reduced chloridazon was tested. When data on non-reduced chloridazon were available they indicated generally comparable results, with the exception of an acute oral toxicity study in rats in which the non-isomer reduced material was somewhat more toxic.

The acute oral toxicity of isomer reduced chloridazon in rats is low. Female rats are somewhat more sensitive (LD_{50} 2140 mg/kg body weight) than males (> 3830 mg/kg body weight). Chloridazon with higher isomer content seems to be slightly more toxic after acute oral exposure to rats, however a different rat strain (Sprague-Dawley oppose to Wistar) might have also influenced the results (LD_{50} >1000 <1470 mg/kg body weight). The LD_{50} of both sexes is comparable. In Shermann rats a difference in LD_{50} values between males and females was observed, resulting in a lower LD_{50} value for female rats (647 mg/kg for adult females vs 1311). This lower value was most likely due to the use of a different carrier when compared to the other studies. The acute oral LD_{50} in mice was determined to be 605 mg/kg bw for males and 598 mg/kg for females.

Chloridazon, whether isomer reduced or isomer rich is virtually non toxic to Wistar or CRJ-SD rats after dermal application. The LD_{50} values were above 2000 mg/kg body weight causing neither mortality nor systemic toxicity. In addition, no local reaction has been observed in these studies at the application site.

The inhalation toxicity of aerosols of chloridazon, both isomer reduced and isomer rich, is low (LC_{50} > 5.4 or > 30.8 mg/L/4 h, respectively). There was only a slight irritation of the airways during exposure in the dust aerosol study. Minor differences in clinical signs in the two inhalation studies might be due to differences in the exposure techniques and the fact that a different rat strain was used.

Isomer reduced chloridazon was not irritant in a skin irritation study in rabbits. A comparison to non isomer reduced chloridazon is not possible as there is only a Draize Test available with significantly different exposure conditions (occlusive dressing, 6 times longer exposure, intact and abraded skin) which would not allow a comparison. Due to the outdated experimental design this study was rejected. Both - isomer reduced and non-reduced forms - of chloridazon are non-irritant to the rabbit eye. Isomer reduced chloridazon is not a skin sensitiser in the Guinea Pig Maximisation Test.

The results of the acute toxicity data of chloridazon are summarised in the table below [see Table B.6.2-1].

Table B.6.2-1: Acute toxicity of chloridazon

Author/Year/ Laboratory/Doc No.	Study type Species/strain	Results LD_{50} in mg/kg LC_{50} in mg/L/4h	Comments
[1988/0445; Gamer A. O., Kirsch P.; 1988a] [redacted]	LD_{50} oral Wistar rat	> 3830 males = 2140 females about 2200 males and females	Isomer reduced. Mortality: 2 at 1210 mg/kg bw 4 at 2260 mg/kg bw 3 at 3830 mg/kg bw
[1985/291; Jaeckh R., Gelbke H.-P., 1981] [redacted]	LD_{50} oral Sprague-Dawley rats	1327 males 1212 females > 1000 < 1470 males and females	Mortality: 1 at 1000 mg/kg bw 8 at 1470 mg/kg bw 9 at 6810 mg/kg bw
[1986/10369; Gaines T. B., Linder R.E.; 1986] Publication	LD_{50} oral Sherman rats	1311 adult males* 647 adult females* 736 weanlings (females)*	* vehicle peanut oil
[1979/10098; Toyoshima S. et al.; 1979a] [redacted]	LD_{50} oral CRJ-ICR mice	605 males 598 females 624 males and females	-
[1988/0469; Gamer A. O., Kirsch P.; 1988c] [redacted]	LD_{50} dermal Wistar rat	> 2000 males and females	Isomer reduced No systemic toxicity No local irritation
[1979/10099; Toyoshima S. et al.; 1979b] [redacted]	LD_{50} dermal CRJ-SD rats	> 5000 males and females	No systemic toxicity No local irritation
[1989/0405; Gamer A. O.; 1989] [redacted]	LC_{50} inhalative Wistar rats	> 5.4 (dust aerosol)	Isomer reduced. No mortalities MMAD 4.0 µm
[1980/042; Leuschner F.; 1980] [redacted]	LC_{50} inhalative Sprague-Dawley rats	> 30.8 (liquid aerosol)	No symptoms No mortalities MMAD 5.4µm
[1989/0101; Kirsch P., Hildebrand B.; 1989a] [redacted]	Primary skin irritation Rabbit White Vienna	Not irritating	Isomer reduced
[1989/0102; Kirsch P., Hildebrand B.; 1989b]; [redacted]	Primary eye irritation Rabbit White Vienna	Not irritating	Isomer reduced
[1984/124; Hildebrand B.; 1984] [redacted]	Primary eye irritation Rabbit New Zealand White	Not irritating	-
[1988/0464; Gamer A. O., Kirsch P.; 1988b]; [redacted]	Skin sensitisation Guinea pig (GPMT) Maximisation Test	Not sensitising	Isomer reduced Dermal induction: 25 % in aqua dest. Dermal challenge: 10 % in aqua dest.

B.6.2.1 Oral

B.6.2.1.1 Rat

Report: Gamer A. O., Kirsch P. 1988(a)
Reg. No. 13 033: Report on the study of acute oral toxicity on the rat based on OECD and EPA (FIFRA)
[redacted]
unpublished
BASF RegDoc# 1988/0445

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: OECD 401, EPA 81-1

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon isomer reduced)
Batch: N 143
Purity: 94.1 %, brown powder
Test Substance No.: 88/174

Test animals: Wistar rats

Five male and five female Wistar rats per dose group were administered doses from 464 to 3830 mg/kg body weight chloridazon in 0.5 % aqueous CMC (carboxymethyl cellulose) by gavage (for detail of dosing see Table B.6.2-2). Chemical analysis was performed with respect on homogeneity, stability and correctness of the concentration of the test substance preparation. The animals were examined for clinical signs and mortality. All rats that died and the surviving rats at the end of the study (14 days observation period) were examined macroscopically.

Table B.6.2-2: Dosing scheme - acute oral toxicity study in Wistar rats with chloridazon:

Number of rats/sex	Applied dose (mg/kg)	Applied volume (mL/kg)	Concentration (g/100 mL) of chloridazon in 0.5 % CMC
5	3830	10	38.3
5	2200	10	22.00
5	1210	10	12.10
5	464	10	4.64

Findings:

Homogeneity and stability of the test substance were confirmed by analysis.
Homogeneity, stability and correctness of the concentration of the test substance preparations were verified analytically.

Table B.6.2-3: Mortality in the acute oral toxicity study in Wistar rats with chloridazon

Dose (mg/kg bw)	Mortality (no of deaths/no of total rats)			Time of death after dosing
	Males	Females	Combined	
464	0/5	0/5	0/10	-
1210	0/5	2/5	2/10	Day 1
2200	1/5	3/5	4/10	Day 1
3830	0/5	3/5	3/10	Day 1

There were no mortalities 1 hour after dosing, all animals that died were found dead within day 1 of the study. There were no late mortalities.
Clinical symptoms recorded were dyspnea, apathy, staggering, tremors, twitching, spastic gait, piloerection, salivation and poor general state. Most of the symptoms were reversible within 1 day. Piloerection was noted up to day 9 of the study.
Rats that died showed general congestion. There were no gross pathological findings in surviving rats.

Conclusion:
The LD_{50} in Wistar rats was calculated as follows:

Males	> 3830 mg/kg body weight
Females	2140 mg/kg body weight
Males and females	about 2200 mg/kg body weight

Report: Jaeckh R., Gelbke H.-P., 1981
Report on the study of the acute oral toxicity of Chloridzon in the rat
[redacted]
unpublished
BASF RegDoc# 1985/291

GLP: No, studies were conducted prior to the implementation of GLP but are scientifically valid

Guideline: not mentioned, study is similar to OECD guideline 401 (1981) and meets good scientific practice

Deviations: Limited reporting

Acceptability: The study is considered to be supplementary.

Material and Methods:

Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon)
5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone
Purity: technical agent, no further details given
Batch No.: not indicated
Test Substance No.: 80/181

Test animals: Sprague-Dawley rats / WIGA

Five male and five female Sprague-Dawley rats per dose group were administered doses from 316 to 6810 mg/kg body weight chloridazon in 0.5 % aqueous CMC (carboxymethyl cellulose) by gavage (for detail of dosing see Table B.6.2-4). Chemical analysis with respect to homogeneity, stability and correctness of concentration of the test substance preparation was not performed. The animals were examined for clinical symptoms and mortality. All rats that died and the surviving rats at the end of the study (14 days observation period) were examined macroscopically.

Table B.6.2-4: Dosing scheme - acute oral toxicity study in Sprague-Dawley rats with chloridazon

Number of rats/sex	Applied dose (mg/kg)	Applied volume (mL/kg)	Concentration (%) of chloridazon in 0.5 % aqueous CMC
5	316	10	3.16
5	464	10	4.64
5	681	10	6.81
5	1000	10	10.00
5	1470	10	14.70
5	6810	13.6	50.00

Findings:
Clinical symptoms recorded were such as dyspnea, apathy, staggering and poor general state for the dose groups from 464 to 6810 mg/kg body weight. No symptoms occurred in the dose level of 316 mg/kg body weight.
Rats that died showed general congestion and acute dilatation of the heart and slight acute emphysema in the lung. There were no gross pathological findings in surviving rats.

Table B.6.2-5: Mortality in the acute oral toxicity study in Sprague-Dawley rats with chloridazon

Dose (mg/kg bw)	Mortality (no of deaths/no of total rats)			Time of death after dosing (first and last mortality at observation day)
	Males	Females	Combined	
316	0/5	0/5	0/10	-
464	0/5	0/5	0/10	-
681	0/5	0/5	0/10	-
1000	0/5	1/5	1/10	Day 1
1470	4/5	4/5	8/10	Day 1
6810	4/5	5/5	9/10	Day 1

Conclusion:
The LD_{50} in Sprague-Dawley rats in this study was calculated as follows:

Males	1327 mg/kg body weight
Females	1212 mg/kg body weight
Males and Females	> 1000 < 1470 mg/kg body weight

The difference in LD_{50} values compared to chloridazon 'isomer reduced' [see Table B.6.2-2] could be either due to different toxicity/purity of both batches of chloridazon and/or to biological variations and the different rat strains used.

Report: Gaines T. B., Linder R. E., 1986
Acute toxicity of pesticides in adult and weanling rats
Fundamental and applied toxicology, No. 7, 299 - 308
published
BASF RegDoc# 1986/10369

GLP: No, not subject of GLP regulations

Guideline: not mentioned, somewhat similar to OECD 401 (1981)

Deviations: Publication, limited information on study design, purity and batch of the compound tested, no analytical data on the compound

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Test substance was identified as follows:
Pyrazon (former trade name of chloridazon)
5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone
Purity: technical agent, no further details given
Batch No: not indicated
Test animals: Sherman (specific pathogen free) rats
The study examined the LD_{50} values of 57 pesticides in Sherman (specific pathogen free) rats via the oral (gavage) and dermal route. No analytical determination of the test compound preparation with respect to homogeneity, stability and target concentrations were performed. Adult rats (90 days old), as well as weanling rats (4 - 6 weeks of age) were tested for their oral LD_{50}. A minimum of 10 animals per group and 4 dose levels (values not further specified) were used for each LD_{50} determination. The animals received the test compound either suspended or as a solution (based on the physico-chemical properties of the chemical) at a volume of 5 to 10 mL/kg body weight preferably in peanut oil. However other vehicles were also used when indicated. For pyrazon the standard vehicle has to be assumed. For male animals the application volume was increased up to 12 mL/kg. The animals were observed for clinical symptoms over the study period of 14 days. Macroscopic pathological evaluations were not performed.

Findings:
Clinical symptoms were not reported. The results were given only in tabulated form with respect to LD_{50} values. Since no other vehicle is indicated for Pyridazon (former trade name of

chloridazon) it has to be assumed that the LD_{50} in adult males (1311 mg/kg body weight), the LD_{50} in females (647 mg/kg body weight) and weanlings (736 mg/kg body weight, only females tested here) was performed in peanut oil (standard vehicle of the study).

Discussion:
The authors conclusion that weanlings are more susceptible to chloridazon than adult rats cannot be followed. When comparing LD_{50} values the vehicle should also be taken into consideration. Peanut oil may lead to a better resorption from the gastrointestinal tract than acetone or aqueous CMC suspension as performed in other acute oral studies with chloridazon. Thus the lower LD_{50} value for females in this study can be explained by the use of a different carrier when compared to other studies. In comparison to the males of this study there were differences in the reported application volumes, which may eventually also have an impact on the oral absorption and thereof the LD_{50} values. In this respect, it is noteworthy that the LD_{50} value in adult females and weanlings are comparable. Due to the questionable methodological procedures and the limited data, this study should not be used for risk assessment purposes.

Conclusion:
The LD_{50} in Sherman rats was reported as follows:

Adult males	1311 mg/kg body weight
Adult females	647 mg/kg body weight
Weanling rats	736 mg/kg body weight

B.6.2.1.2 Mouse

Report: Toyoshima S. et al., 1979(a)
Acute oral, subcutaneous and intraperitoneal toxicity studies of Pyramin in the mouse
[redacted]
unpublished BASF
RegDoc# 1979/10098

GLP: No, studies were conducted prior to the implementation of GLP but are scientifically valid

Guideline: not mentioned, study is similar to OECD Guideline 401 and meets good scientific practice

Deviations: No analytical data on the test compound

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Test substance was identified as follows:
Pyramin (former trade name of chloridazon)
5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone
Purity: 86.6 % - technical agent,
Lot No. 3.11.78 provided by BASF Japan
Test animals: CRJ-ICR mice

Ten male and ten female CRJ-ICR mice per dose group were administered doses from 400 to 976 mg/kg body weight chloridazon in corn oil by gavage (for detail of dosing see Table B.6.2-6). Analytical examinations of the test substance preparations were not performed in this study. The animals were examined for clinical signs and mortality. All mice that died and the surviving mice at the end of the study (14 days observation period) were examined macroscopically.

Table B.6.2-6: Dosing scheme - acute oral toxicity study in CRJ-ICR mice with chloridazon

Number of rats/sex	Applied dose (mg/kg)	Applied volume (mL/kg)
10	400	5
10	500	5
10	625	5
10	781	5
10	976	5

Findings:

Table B.6.2-7: Mortality in the acute oral toxicity study in CRJ-ICR mice with chloridazon

Dose (mg/kg bw)	Mortality (no of deaths/no of total rats)			Time of death after dosing (first and last mortality at observation day)
	Males	Females	Combined	
400	0/10	0/10	0/20	
500	3/10	2/10	5/20	Within 6h after dosing
625	3/10	5/10	8/20	Within 6h after dosing
781	8/10	9/10	17/20	Within 6h after dosing
976	10/10	10/10	20/20	Within 6h after dosing

All mortalities occurred within 6 hours after test substance administration. Clinical symptoms recorded were various and unspecific and returned to normal within 1 day in surviving mice. There were no gross pathological findings in mice that died or in surviving mice.

Conclusion:
The LD_{50} in CRJ-ICR mice was calculated as follows:

Males	650 (565 - 748) mg/kg body weight
Females	598 (512 - 698) mg/kg body weight
Males and Females	624 mg/kg body weight

Chloridazon has a very fast onset of toxicity and recovery and a relatively steep dose response curve in CRJ-ICR mice. There is no sex specific difference.

B.6.2.2 Percutaneous

Report: Gamer A. O., Kirsch P., 1988(c)
Reg. No. 13 033: Report on the study of acute dermal toxicity on the rat based on OECD and EPA (FIFRA)
[redacted]
unpublished BASF
RegDoc# 1988/0469

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: OECD 402, EPA 81-2

Deviations: Limited data reporting, no individual animal records, analytical data on test compound referred to in study 10A0174/881116. Data can be requested from the applicant.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon isomer reduced)
5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone
Purity: 94.1 % brown powder
Batch No.: N 143
Test Substance No.: 88/174
Test animals: Wistar rats

Five male and five female Wistar rats were treated with the test substance prepared in 0.5 % aqueous CMC which was applied to about 50 cm^2 on the dorsal flank under semi occlusive conditions for 24 hours. The test substance concentration in the vehicle was 50 g/100mL (w/v) and the application volume was 4 mL/kg. After 24 hours of exposure the treated skin was cleaned with water. Analytical determinations of the test substance preparation (stability and homogeneity, verification of dose level) were not performed. The animals were examined for clinical signs and mortality. All rats that died and the surviving rats at the end of the study (14 days observation period) were examined macroscopically.

Findings:
Homogeneity and stability of the test substance was reported to be confirmed by analysis. However, the analytical data were not provided.

Table B.6.2-8: Mortality, systemic/local toxicity in the acute dermal toxicity study in Wistar rats with chloridazon

Dose (mg/kg bw)	Mortality (no of deaths/no of total rats)			Symptoms
	Males	Females	Combined	
2000	0/5	0/5	0/10	Systemic: none Local skin reaction: none

There were no signs of systemic intoxication or mortalities or local signs of irritation. No macroscopic findings were noted at sacrifice.

Conclusion:
The dermal LD_{50} in rats was found to be > 2000 mg/kg bw for male and female animals.

Report:	Toyoshima S. et al., 1979(b) Acute dermal toxicity studies of Pyramin in the rat [redacted] unpublished BASF RegDoc# 1979/10099
GLP:	No, studies were conducted prior to the implementation of GLP but are scientifically valid
Guideline:	not mentioned, study is similar to OECD 402 and meets good scientific practice
Deviations:	Body weight too low, two dose groups only, very high doses of 3000 and 5000 mg/kg bw, no information whether the substance was applied as solution or as powder to moistened skin
Acceptability:	The study is considered to be supplementary.

Material and Methods:
Test material: Test substance was identified as follows:
Pyramin (former trade name of chloridazon)
5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone
Purity: 86.6 % - technical agent, brown powder
Lot No. 3.11.78 provided by BASF Japan
Test animals: CRJ-SD rats

Ten male and female CRJ-SD rats were treated with 2 dose levels of the test substance (3000 and 5000 mg/kg bw), which was applied unchanged, moistened with water to about 20 cm^2 on the dorsal flank under semi occlusive conditions for 24 hours. After 24 hours of exposure the treated skin was cleaned with water. Analytical determinations of the test substance preparation (stability and homogeneity, verification of dose level) were not performed. The animals were examined for clinical signs and mortality. All surviving rats at the end of the study (14 days observation period) were examined macroscopically.

Findings:

Table B.6.2-9: Mortality, systemic/local toxicity in the acute dermal toxicity study in CRJ-SD rats with chloridazon

Dose (mg/kg bw)	Mortality (no of deaths/no of total rats)			Symptoms
	Males	Females	Combined	
3000	0/10	0/10	0/20	Systemic: none Local skin reaction: none
5000	0/10	0/10	0/20	Systemic: none Local skin reaction: none

Discussion:
As the study does not correspond to the guideline it does not provide reliable data. Therefore, the study should not be used in risk assessment.

Conclusion:
Under the conditions of the study the dermal LD_{50} in rats was found to be > 5000 mg/kg bw for male and female animals.

B.6.2.3 Inhalation

Report: Gamer A. O., 1989
Study on the acute inhalation toxicity LC_{50} of Reg. No. 13 033; isomer reduced as a dust aerosol in rats - 4-hour exposure
[redacted]
unpublished BASF
RegDoc# 1989/0405

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: OECD 403, EPA 81-2

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon – isomer reduced)
5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone
Purity: at least 94.1 % - solid brown
Batch No: N 143
Test Substance No.: 88/174-1
Test animals: Wistar rats

Five male and female Wistar rats per dose group were exposed to dust aerosols with head-nose exposure to concentrations of 0 (control) and 5.4 mg/L chloridazon in the air for a period of 4 hours (for details see Table B.6.2-10). The concentrations were verified analytically. The

MMAD 50 % (mass median aerodynamic diameter 50 %) and the fraction of respirable dust were determined. During and after exposure behaviour and general conditions were recorded. Body weight was determined once a week. All rats were subjected to gross pathology 14 days after exposure.

Table B.6.2-10: Experimental procedure LC_{50} determination in Wistar rats with chloridazon

Dose group	Analytical concentration (mg/L)	Number of animals
1 (control)	0	5 males and 5 females
2	5.4	5 males and 5 females

Findings:
Homogeneity and stability of the test substance were confirmed by analysis.

Table B.6.2-11: Analytical concentration and mortalities in the LC_{50} head nose determination in Wistar rats with chloridazon (dust aerosol)

Dose group	Concentration in mg/L		Mortality (dead animals/animals exposed)	
	Analytical	Nominal	Males	Females
1 (control)	0	0	0/5	0/5
2	5.4	45.3	0/5	0/5

The MMAD 50 % was determined to be 4.0 µm with a geometrical standard deviation of 3.1. Although 1 % aerosol (mixed with the test substance) was used, no smaller particle size could be generated. The amount of respirable dust that might reach the alveolar region was calculated to be 79 %.
During exposure to chloridazon irregular and accelerated respiration and closure of the eye lids were noted. There were no clinical symptoms after exposure and no mortalities occurred. Body weight in females was comparable to historical control data. In male rats, there was a retardation of body weight in the second week of the observation period. No pathological findings were noted at study termination (day 14).

Conclusion:
Chloridazon has a low acute inhalation toxicity as dust aerosol. The 4-hour LC_{50} by head-nose exposure was calculated to be:

> 5.4 mg/L (male and female rats)

Report: Leuschner F., 1980
The acute toxicity of test compound Reg. No. 13 033 (chloridazon) when administered to rats by inhalation
[redacted]
unpublished BASF
RegDoc# 1980/042

GLP: No, studies were conducted prior to the implementation of GLP but are scientifically valid

Guideline: Experimental method: Principles and procedures for evaluating the toxicity of household substances, publication 1138 National Academy of Sciences National Research Council Washington (1964), page 14 – 20
In accordance with OECD 403

Deviations: Purity, batch not identified, no analytical data on the test compound and aerosol concentrations provided.

Acceptability: The study is considered to be acceptable as supplementary.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon)
5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone
Purity: technical agent – dark red-brown solid
Batch No.: not indicated
Test animals: Sprague Dawley rats

Ten male and female Sprague Dawley rats per dose group were exposed to an aerosol (1.2 g chloridazon was solved in 100 mL ethanol) by head-nose exposure for 4 hours. The concentrations ranged from 0 (control) to 30.8 mg/L in the air (for details see Table B.6.2-12). The concentrations were verified analytically. During and after exposure behaviour and general conditions were recorded. Body weight was determined once a week. All rats were subjected to gross pathology after a 14 day observation period.

Table B.6.2-12: Experimental procedure LC_{50} determination in Sprague Dawley rats with chloridazon

Dose group	Nominal Concentration (mg/L)	Analytical Concentration (mg/L)	Number of animals
1 (control)	0	0	10 males and 10 females
2	92.7	9.6	10 males and 10 females
3	165.8	18.9	10 males and 10 females
4	184.2	30.8	10 males and 10 females

Findings:

Table B.6.2-13: Symptoms and mortalities in the LC_{50} head nose determination in Sprague-Dawley rats with chloridazon

Dose group	Analytical concentration (mg/L/4h)	Mortalities	Symptoms /effects on body weight / macroscopic findings
1 (control)	0	0/10 males 0/10 females	None
2	9.6	0/10 males 0/10 females	None
3	18.9	0/10 males 0/10 females	None
4	30.8	0/10 males 0/10 females	None

The MMAD 50 % (mass median aerodynamic diameter 50 %) was 5.4 µm with a standard deviation of 1.9. A total of 90 % of the droplet size was below 10 µm.
There were no clinical symptoms during and after exposure of chloridazon. There was also no effect on body weight and no findings were noted at macroscopic examination on day 14 of the study.

Conclusion:
The LC_{50} in Sprague Dawley rats after 4 hour head-nose exposure of a liquid aerosol of chloridazon was as follows:

male and female rats > 30.8 mg/L/4h

B.6.2.4 Skin irritation

Report: Kirsch P., Hildebrand B., 1989(a)
Reg. No. 13 033: Report on the acute dermal irritation/corrosivity to the intact dorsal skin of the white rabbit based on OECD and EPA (FIFRA)
[redacted]
unpublished BASF
RegDoc# 1989/0101

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: OECD 404, EPA 81-5

Deviations: None

Acceptability: The study is considered to be acceptable

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon - isomer reduced)
5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone
Purity: 94.1 % brown powder
Batch No.: LV 143
Test Substance No.: 88/174
Test animals: White Vienna rabbits

Chloridazon was tested for skin irritation/corrosion in White Vienna rabbits. About 0.5 g of unchanged chloridazon moistened with water was given onto a shaven area of 2.5 x 2.5 cm of the upper third of the back or flanks of 6 rabbits (3 males and 3 females) for 4 hours under semi-occlusive dressing. At the end of the exposure period the test substance was removed with lutrol and lutrol/water (1:1). The untreated skin served as negative control. Evaluation of the skin was performed 24, 48 and 72 hours after test substance application.

Findings:
Homogeneity and stability of the test substance were confirmed by analysis.

Table B.6.2-14: Skin irritation results of chloridazon in White Vienna rabbits

Animal No.	1	2	3	4	5	6
	R/ED*	R/ED	R/ED	R/ED	R/ED	R/ED
24h	1/0	1/0	1/0	1/0	1/0	1/0
48h	1/0	0/0	0/0	0/0	1/0	0/0
72h	0/0	0/0	0/0	0/0	0/0	0/0
Mean value	0.3/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.3/0.0	0.0/0.0

*R/ED = redness/oedema

No oedemas were noted in any rabbit at any time. Slight erythema (grade 1) was observed in all animals 1 day after the exposure period. This finding persisted until day 2 after exposure in two out of six rabbits. Afterwards there were no findings, thus the study was terminated 72 hours after exposure.
The total mean values were 0.1 for redness and 0.0 for oedema.

Conclusion:
Chloridazon is non irritant to the rabbit skin under the test conditions chosen.

B.6.2.5 Eye irritation

Report: Kirsch P., Hildebrand B., 1989(b)
Reg. No. 13 033: Report on the acute irritation to the eye of the white rabbit based on OECD and EPA (FIFRA)
[redacted]
unpublished
BASF RegDoc# 1989/0102

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: OECD 405, EPA 81-4

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon - isomer reduced)
5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone
Purity: 94.1 % brown powder
Batch No: N 143
Test Substance No.: 88/174
Test animals: White Vienna rabbits

A volume of 0.1 mL of the unchanged test substance was instilled into the right eye of four male and two female White Vienna rabbits. The animals were scored for corneal changes, iris effects and conjunctival reaction, 24, 48 and 72 hours after test substance administration (study termination).

Findings:
Homogeneity and stability of the test substance were confirmed by analysis.

Table B.6.2-15: Results of the primary eye irritation test in rabbits

Animal/time of examination	Cornea		Iris	Conjunctivae		
	Opacity	Affected area	Injury grade	Redness	Chemosis	Discharge
1 h						
Animal 1	0	0	0	2	0	1
Animal 2	0	0	0	2	0	1
Animal 3	0	0	0	2	0	1
Animal 4	0	0	0	2	0	1
Animal 5	0	0	0	2	0	1
Animal 6	0	0	0	2	0	1
24 h	0					
Animal 1	0	0	0	1	0	0
Animal 2	0	0	0	1	0	0
Animal 3	0	0	0	1	0	0
Animal 4	0	0	0	0	0	0
Animal 5	0	0	0	1	0	0
Animal 6	0	0	0	1	0	0
48 h						
Animal 1	0	0	0	0	0	0
Animal 2	0	0	0	0	0	0
Animal 3	0	0	0	0	0	0
Animal 4	0	0	0	0	0	0
Animal 5	0	0	0	0	0	0
Animal 6	0	0	0	0	0	0
72 h						
Animal 1	0	0	0	0	0	0
Animal 2	0	0	0	0	0	0
Animal 3	0	0	0	0	0	0
Animal 4	0	0	0	0	0	0
Animal 5	0	0	0	0	0	0
Animal 6	0	0	0	0	0	0
Mean	0.0		0.0	0.3	0.0	

There were no effects on cornea and iris in this study. Shortly after instillation grade 2 redness and discharge grade 1 of the conjunctivae was noted. After 24 hours only grade 1 redness was observed. After 48 and 72 hours no such findings were recorded, thus the study was terminated after 72 hours.

Discussion:
Classification not irritating.

Conclusion:
Chloridazon is not irritant to the eyes of White Vienna rabbits under the experimental conditions chosen.

Report: Hildebrand B., 1984
Study of the primary irritation of 'Reg. No. 13 033 (pyrazon)' to the eye of white rabbits
[redacted]
unpublished
BASF RegDoc# 1984/124

GLP: No, studies were conducted prior to the implementation of GLP but are scientifically valid

Guideline: according to the method given in Federal Register 38, No. 187, § 1500.42, P. 27019, Sept. 27, 1973 and appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, FDA, Austin, Texas, 1959

Deviations: Guideline not available. Very limited reporting on the study design. No information on purity and batch of the test compound.

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon, pyrazon)
1-phenyl-4-amino-5-chloro-pyridazone-(6)
Purity: not indicated
Batch No.: not indicated
Test Substance No.: 77/38
Test animals: New Zealand White rabbits

The test substance (50 mg/animal) was instilled into the right eye of four male and two female New Zealand White rabbits. The animals were scored for corneal changes, iris effects and conjunctival reaction 24, 48, 72 hours and 8 days after test substance administration.

Findings:

Table B.6.2-16: Results of the primary eye irritation test in New Zealand White rabbits

Animal/time of examination	Córnea		Iris	Conjunctivae		
	Opacity	Affected area	Injury grade	Redness	Chemosis	Discharge
24 h						
Animal 1	0	0	0	1	0	1
Animal 2	0	0	0	1	0	1
Animal 3	0	0	0	1	0	0
Animal 4	0	0	0	1	0	0
Animal 5	0	0	0	1	0	1
Animal 6	0	0	0	1	0	0
48 h						
Animal 1	0	0	0	1	0	1
Animal 2	0	0	0	1	0	0
Animal 3	0	0	0	1	0	0
Animal 4	0	0	0	1	0	0
Animal 5	0	0	0	1	0	0
Animal 6	0	0	0	1	0	0
72 h						
Animal 1	0	0	0	1	0	0
Animal 2	0	0	0	1	0	0
Animal 3	0	0	0	1	0	0
Animal 4	0	0	0	0	0	0
Animal 5	0	0	0	1	0	0
Animal 6	0	0	0	2	0	0
8 days						
Animal 1	0	0	0	1	0	0
Animal 2	0	0	0	0	0	0
Animal 3	0	0	0	1	0	0
Animal 4	0	0	0	0	0	0
Animal 5	0	0	0	0	0	0
Animal 6	0	0	0	0	0	0

PII (Primary Irritation Index) = 2.44

There were no effects on cornea and iris in this study. Conjunctival effects were restricted mainly to grade 1 erythema in most of the animals after 1, 2 or 3 days. Reversibility was noted after day 8 when only two animals had a slight redness. In addition a slight discharge was noted in three rabbits after one day and one animal after two days.

Conclusion:
Chloridazon is not irritant to the eyes of New Zealand White rabbits under the experimental conditions chosen.

B.6.2.6 Skin sensitisation

Report: Gamer A. O., Kirsch P., 1988(b)
Report on the maximisation test for the sensitising potential of Reg. No. 13 033; isomer reduced in guinea pigs
[redacted]
unpublished
BASF RegDoc# 1988/0464

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: OECD 406

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon – isomer reduced)
5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone
Purity: 94.1 % brown powder
Batch No.: N 143
Test Substance No.: 88/174-1
Test animals: Guinea Pig

Twenty animals per test group and control group were tested in the Guinea Pig Maximisation Test (GPMT) according to the method of Magnusson and Kligman. The study conditions can be derived from the following table. The doses were selected upon the results of a preliminary study where 25 % aqueous chloridazon was the minimum irritant concentration and 10 % aqueous chloridazon the maximum non-irritant concentration after two 24 hours percutaneous occlusive applications. The sensitivity and the reliability of the experimental technique was proved with dinitrochlorobenzene as a positive control in a separate study.

Findings:

Table B.6.2-17: Test conditions - Guinea Pig Maximisation Test (GPMT)

Intradermal induction (day 0)	**5 % in aqua dest.**
Dermal induction (one week after intradermal induction)	25 % in aqua dest.
1st Dermal challenge (two weeks after dermal induction)	10 % in aqua dest.
2nd Dermal challenge (one week after first dermal challenge)	10 % in aqua dest.

The intradermal induction caused distinct erythema and oedema formation. After dermal induction distinct erythema and incrustation, partially open (caused by intradermal induction) were observed in addition to distinct oedema in the test group. Control animals were not

treated, as the vehicle (Aqua dest.) was not expected to influence the test results. The results are summarised in Table B.6.2-18.

Table B.6.2-18: Results - Guinea Pig Maximisation Test (GPMT)

Challenge	1st challenge	2nd challenge
Test substance concentration	10 % in aqua dest.	10 % in aqua dest.
Control group 1	0/10	0/10
Control group 2	No application of test substance	0/10
Test group	0/20	0/20

X/Y: number of positive reactions/number of animals tested (reading 48 h after beginning of test substance application)

There was no skin reaction in control and test group animals indicating that there was no sensitising effect.

Conclusion:
Chloridazon does not have a sensitising effect on the skin of guinea pig in the Maximisation Test under the test conditions chosen.

B.6.3 Short-term toxicity (Annex IIA 5.3)

The 4-week short term oral toxicity of chloridazon was investigated in rats and dogs. For both species a dose level of 10000 ppm proved to be too high (animals in moribund condition).
To determine whether there were differences in toxicity between both qualities of chloridazon, chloridazon isomer rich (original lower purity) and isomer reduced (higher purity) were tested comparitively in two 4-week feeding studies in Wistar rats. A dose of 5000 ppm in rats resulted in reduced food consumption, body weight and slight clinical chemical changes (e.g. increased cholesterol values). Target organs were the kidneys and liver. There were no major differences between the two qualities tested.

Beagle dogs dosed up to 2700 ppm did not demonstrate test substance related effects. At 5000 ppm reduced food consumption and body weight development were noted. There was a slight anemic effect in females. A few clinical chemical parameters (e.g. increased cholesterol) were affected. The target organs were the liver and the kidneys.

The 4-week toxicity of chloridazon is comparable in rats and dogs. The NOAEL in dogs is 81.9 mg/kg bw, in rats the NOAEL is 40 mg/kg bw. The latter value, however, is the result of wide spacing of the dose levels (500 ppm as NOAEL and 5000 ppm as LOAEL).

The subchronic toxicity of chloridazon was studied in 90-day studies in rats, mice and dogs.

In Sprague-Dawley rats a dose of 19200 ppm proved to be too high and was reduced to 9600 ppm after 5 weeks. At this dose and at 4800 ppm reduced food consumption and body weight gain was observed. Reduced erythrocyte and hemoglobin values in females and altered clinical chemical changes (indicating an impairment of liver function) were observed at the high dose level only. The target organ was the liver: increased weight (4800 ppm and higher – both sexes and 1200 ppm females), hepatocyte enlargement, decreased glycogen content (4800 ppm and higher). The NOAEL was 300 ppm (21 mg/kg bw).

In B6C3F1 mice reduced food consumption and body weight was noted at 7500 ppm. Body weight was also reduced in 1500 ppm males. At 1500 ppm and higher reduced triglyceride and cholesterol values were seen in males. The target organ was the liver: increased weight (7500 ppm both sexes, 1500 ppm males). The NOAEL was 300 ppm in males (65 mg kg/bw) and 1500 ppm in females (467 mg/kg bw).

In Beagle dogs three 90-days studies were carried out. Toxicity was observed at dose levels of 4000 ppm and higher. Toxicity consisted of reduced body weight gain, reduced protein values, suggesting impaired liver function. The target organs were the liver (increased weight) and the kidneys (increased weight – males and vacuolisation of distal renal tubules – females). The overall NOAEL was established at 3000 ppm (97 mg/kg bw).

The 12-month oral toxicity of chloridazon was studied in beagle dogs. Toxicity was observed at dose levels of 1200 ppm and higher. Toxicity consisted of slightly reduced food consumption in both sexes (6000, 8000 ppm) and reduced body weight gain in females (1200, 3600 ppm), increased inorganic phosphate in both sexes (6000, 8000 ppm) and reduced bilirubin in males (8000 ppm). Target organs were the kidneys and the gastric mucosa, possibly due to local irritation. The LOEL in dogs is 1200 ppm and the NOAEL 400 ppm equivalent to 11 mg/kg bw.

In conclusion, the short-term oral toxicity of chloridazon was characterised by effects on the body weight and the liver in all three species tested. At high dose levels liver function was impaired resulting in clinical chemical changes, whereas at lower dose levels only liver weight increases were seen. Kidney toxicity and effects on the gastric mucosa were observed in dogs at very high dose levels.

In a 21-day dermal study in New-Zealand White rabbits neither systemic toxicity nor signs of local irritation were observed at a dose level of 1000 mg/kg bw, indicating the very low toxic potential of the test substance after dermal exposure.

Based on the above-described findings, the relevant NOAELs from the short-term toxicity studies are listed below.

Table B.6.3-1: NOAEL's from short-term studies in different species (mg/kg bw)

Species	Route	Males	Females
Rat	oral – 4 weeks	40	40
Dog	oral – 4 weeks	81.9	81.9
Rat	oral – 3 months	21	23.5
Mouse	oral – 3 months	65	467
Dog	oral – 3 months	97	97
Dog	oral - 12 months	11	11
Rabbit	dermal – 3 weeks	1000	1000

Table B.6.3-2: Summary results from short-term studies with chloridazon

Institute/year/ reference	Study type/ species/ dose levels	Comments	NOAEL
[1993/11974; Kirsch P., Hildebrand B.; 1993a] [redacted]	4 week dietary study Wistar rat 0; 500; 10000 ppm Comparative study with two different qualities of chloridazon	10000 ppm: Lethal for many animals. No obvious differences between the two qualities. Assessment of chloridazon specific toxicity not possible due to moribund condition of the animals.	500 ppm (40 mg/kg bw)
[1993/11975; Kirsch P., Hildebrand B.; 1993b] [redacted]	4 week dietary study Wistar rat 0; 5000 ppm Comparative study with two different qualities of chloridazon	5000 ppm: Reduced food consumption and body weight. Slight clinical chemical changes. Target organs: kidneys and liver No differences between the two qualities of chloridazon.	< 5000 ppm (435 mg/kg bw – males) (450 mg/kg bw – females)
[1991/10437; Hellwig J., Kirsch P.; 1991] [redacted] AND [1991/11248; Hellwig J., Hildebrand B.; 1991] [redacted]	4 week dietary range-finding study Beagle dogs 0; 900; 2700; 5000 and 10000 ppm	10000 ppm: Animals in moribund conditions killed prematurely (day 7) 5000 ppm: Vomiting, reduced food consumption and body weight gain. Reduced red blood cell values for one female. Altered clinical chemical values. Target organs: Kidneys: reduced weight, vacuolisation in distal tubules (females) Liver: hepatocellular vacuolisation	2700 ppm (81.9 mg/kg bw)
[1992/10413; Kirsch P., Hildebrand B.; 1992] [redacted]	21 day dermal study New Zealand White rabbits 0 and 1000 mg/kg bw	No systemic toxicity. No signs of local irritation	1000 mg/kg bw

Institute/year/ reference	Study type/ species/ dose levels	Comments	NOAEL
[1975/028; Hunter B. et al.; 1975] [redacted]	90 day dietary study Sprague Dawley rats 0, 300, 1200, 4800 and 19200 ppm The 19200 ppm dose level was reduced to 9600 pm after the 5th week of administration.	19200 / 9600 ppm: Several animals in sacrificed prematurely because of retardation of growth, emaciation or loss of use of hind limbs 19200 / 9600 and 4800 ppm: Reduced food consumption and body weight gain. 19200 / 9600 ppm: Reduced erythrocyte and hemoglobin values in females, altered clinical chemical changes. Target organ: Liver: increased weight (4800 ppm and higher – both sexes and 1200 ppm females), centrolobular hepatocyte enlargement, decreased glycogen content (4800 ppm and higher)	300 ppm (20.7 mg/kg bw – males) (23.5 mg/kg bw – females)
[1990/0568; Schilling K., Hildebrand B.; 1990] [redacted]	90 day dietary study B6C3F1 mice 0; 300; 1500 and 7500 ppm	7500 ppm: Reduced food consumption and body weight. Body weight also reduced in 1500 ppm males. 1500 ppm and higher: Reduced triglyceride and cholesterol values in males. Target organ: Liver: increased weight (7500 ppm both sexes, 1500 ppm males)	300 ppm – males (65 mg/kg bw) 1500 ppm – females (467 mg/kg bw)
[1975/029; Leuschner F. et al.; 1975] [redacted]	90 day dietary study Beagle dogs 0; 100; 300; 900 and 10000 ppm	10000 ppm: Reduced body weight gain, reduced protein values. Altered organ weights, related to the lower body weights.	900 ppm (28.8 mg/kg bw)
[1992/11648; Hellwig J., Hildebrand B.; 1992] [redacted]	90 day dietary study Beagle dogs 0; 300; 1000 and 3000 ppm	No test substance related effects.	3000 ppm (97 mg/kg bw)

Institute/year/ reference	Study type/ species/ dose levels	Comments	NOAEL
[1993/10823; Mellert W.; 1993] [redacted]	90 day dietary study Beagle dogs 0; 4000 and 5000 ppm	4000 and 5000 ppm: Slightly reduced food consumption and body weight gain (females). Target organs: Liver: increased weight (males, 4000 and 5000 ppm) Kidneys: increased weight (males, 5000 ppm), vacuolisation distal renal tubules (females, both doses)	< 4000 ppm (133 mg/kg bw)
[1993/10815; Mellert W. et al.; 1993a] [redacted]	12 month feeding beagle dogs 0, 400, 1200 and 3600 ppm	Reduced body weight gain at 1200 and 3600 ppm	400 ppm 11 mg/kg bw
[1993/10824; Mellert W. et al.; 1993d] [redacted]	12 month feeding beagle dogs 0, 6000 and 8000 ppm	6000 and 8000 ppm: Slightly reduced food consumption in both sexes. Slight reduced body weight gain in females. 8000 ppm: Slightly reduced body weight gain in females. 6000 and 8000 ppm: Increased inorganic phosphate in both sexes. 8000 ppm: Reduced bilirubin in males. Target organs: Kidneys, Gastric mucosa possibly due to irritation.	< 6000 ppm < 186 mg/kg bw

B.6.3.1 Oral administration (28-day studies)

B.6.3.1.1 Rat

Report: Kirsch P., Hildebrand B., 1993 (a)
Study of the oral toxicity of Reg. No. 13 033 in Wistar rats. Administration in the diet over 4 weeks (comparative study of test substances Nos. 86/98 and 86/99)
[redacted]
unpublished
BASF RegDoc# 1993/11974

GLP: Yes (laboratory certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 31 80, 6500 Mainz 1)

Guideline: OECD 407

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: The test substances, chloridazon of different purity, 1) a new batch from a new manufacturing process containing less iso-chloridazon and a high purity and 2) the previous material, were identified as follows:
1. Reg. No. 13 033 (chloridazon), solid, yellow-beige
   Purity: 95.3 %, isomer reduced
   Batch No.: K50/16 A
   Test substance No.: 86/99
2. Reg. No. 13 033 (chloridazon), solid, yellow-beige
   Purity: 84.3 %
   Batch No.: N 121
   Test substance No.: 86/98

Test animals: Wistar rats

The purpose of this study was to compare the short-term toxicity elicited by two different qualities of chloridazon in rats.
Chloridazon was administered to five male and five female Wistar rats per dose level and study group, via their diet in different degrees of purity (84.3 % and 95.3 %) in doses of 0; 500 and 10000 ppm for 4 weeks.
Feed consumption and body weight were determined once a week, as was drinking water consumption from the 2nd week after the beginning of administration onward. The state of health of the animals was checked each day, and when the animals were weighed, they were also inspected and palpated.
29 days after the beginning of administration, blood samples were taken for clinic chemical and hematological examinations.
At the end of the 4-week administration period, all surviving animals were assessed by gross pathology. Subsequently, a histopathological examination was carried out.

Findings:
The stability of the test substances was demonstrated. The homogeneity and the correctness of the concentration in the food were analytically verified. The stability of the test substance (batch N 121, test substance 86/98) in the food was established.
On account of the extremely low feed values in the 10000 ppm groups, the ratio of the amounts of test substance taken in by the animals in the specific dose groups per day (in mg/kg bw) did not correspond to the dose factor selected.
The following test substance related effects were observed at 10000 ppm.
All females treated with the original (No. 86/98) test substance and new material (No. 86/99) died. A total of 3 of 5 males treated with the isomer reduced chloridazon died.
Food and drinking water consumption was reduced for males and females. Body weight gain was markedly reduced in both sexes (males, test group 2: -50 %, test group 4: -67.7 %).

Females of both groups lost weight up to cachexia. Because of the complete mortality body weight was not determined at the end of the study.
Due to the prefinal and moribund state of the animals meaningful data on test substance related effects at 10000 ppm cannot be derived from this study.
At the 500 ppm dose level (approximately 40 mg/kg bw) there were no test substance related effects elicited by either quality of chloridazon.

Conclusion:
The dose level of 10000 ppm proved to be too toxic. There are no apparent differences in the toxicity elicited by either quality of chloridazon. On account of the catabolic state of the animals most of which were in a prefinal stage, a clear differentiation between changes directly induced by the test substance and secondary changes cannot be made at this dose level.

The NOAEL (for either quality of chloridazon) was 500 ppm (approximately 40 mg/kg bw).

Report:	Kirsch P., Hildebrand B., 1993(b) Study of the oral toxicity of Reg. No. 13 033 in Wistar rats. Administration in the diet over 4 weeks (comparative study of test substance Nos. 86/98 and 86/99) [redacted] unpublished BASF RegDoc# 1993/11975
GLP:	Yes (laboratory certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 31 80, 6500 Mainz 1)
Guideline:	OECD 407
Deviations:	One dose group only, complementation to study BASF RegDoc# 1993/11974
Acceptability:	The study is considered to be acceptable as supportive.

Material and Methods:
Test material: The test substances, chloridazon of different purity, 1) a new batch from a new manufacturing process containing less iso-chloridazon and a high purity and 2) the previous material, were identified as follows:

1. Reg. No. 13 033 (chloridazon), solid, yellow-beige
   Purity: 95.3 %, isomer reduced
   Batch No.: K50/16 A
   Test Substance No.: 86/99
2. Reg. No. 13 033 (chloridazon), solid, yellow-beige
   Purity: 84.3 %
   Batch No.: N 121
   Test substance No.: 86/98

Test animals: Wistar rats

The purpose of this study was to compare the short-term toxicity elicited by two different purities of chloridazon in rats. In a previous study [see 1993/11974 Kirsch P., Hildebrand B.,

1993a] the high dose level of 10000 ppm proved to be too toxic to draw definitive conclusions. Therefore, a second study with a lower dose level was performed. The aim of this study was to compare the different qualities of chloridazon, not to obtain a NOAEL (which was already established for both qualities at 500 ppm i.e. 40 mg/kg bw).
Chloridazon was administered to five male and five female Wistar rats per dose level and study group, via their diet in different degrees of purity (84.3 and 95.3 %) in doses of 0 and 5000 ppm for 4 weeks.
Feed and drinking water consumption as well as body weight were determined once a week. The state of health of the animals was checked each day, and when the animals were weighed, they were also inspected and palpated.
At 28 days after the beginning of administration, blood samples were taken for clinicochemical and hematological examinations.
At the end of the 4-week administration period, all surviving animals were assessed by gross pathology. Subsequently, a histopathological examination was carried out.

Findings:
The stability of the test substances was demonstrated. The homogeneity and the correctness of the concentration in the food were analytically verified. The stability of the test substance (batch N 121, test substance 86/98) in the food was established.

Table B.6.3-3: Test substance intake

Dietary dose level (ppm)	Test substance intake males (mg/kg bw)#	Test substance intake females (mg/kg bw)#
5000	435	450

Test substance intake represents the mean of both chloridazon qualities

The following test substance related effects were observed at 5000 ppm:
All females treated with the new test substance and one female treated with the lower purity showed piloerection, a poor general state and cachexia. Most of these animals also demonstrated unphysiological movements and an abnormal position of the toes in each case. An excessive growth of teeth was observed in one animal.
Reduced feed consumption was observed in all treated animals. Drinking water consumption was reduced in the females treated with the higher purity material.
Body weight gain was reduced in males treated with low and high purity material (-10 % and -11.3 % respectively). In the females, body weight gain was considerably impaired (-26.7 % and -37.4 % respectively).
Clinical chemistry revealed the following test substance related changes for either quality of chloridazon:
Significantly increased cholesterol concentrations in the males and females. Reduced creatinine concentrations in the females. Shortened thromboplastin time in the females.
Pathological examinations demonstrated the following test substance related changes for either purity of chloridazon:
Hydropic and vacuolar degeneration of the hepatocytes and decrease of the alimentary fatty deposits in all males and females. Degenerative tubular changes in the kidney in all males and females. In the males these changes were more pronounced than in the females as demonstrated by the fact that they were always necrotising.

Conclusion:
There are no apparent differences in the toxicity elicited by either quality of chloridazon. The target organs for toxicity were the kidneys and the liver.

The NOAEL was below 5000 ppm (approximately 435 (males) – 450 (females) mg/kg bw) but had been established in a previous study [see 1993/10632, Hellwig J.,Hildebrand B., 1993], for both qualities of chloridazon at 500 ppm (40 mg/kg bw).

B.6.3.1.2 Dog

Report: Hellwig J., Kirsch P., 1991
Report on the study of the toxicity of Reg. No. 13 033; 95 % in Beagle dogs. Administration via the diet over 4 weeks (range-finding-study)
[redacted]
unpublished
BASF RegDoc# 1991/10437

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: Range-finding study, comparable to OECD 407 (1981)

Deviations: Comparable to OECD 407

Acceptability: The study is considered to be acceptable.

And

Report: Hellwig J., Hildebrand B., 1991
Amendment No. 1 to the report of June 5, 1991. Study of the toxicity of Reg. No. 13 033; 95 % in Beagle dogs - Administration via the diet over 4 weeks (range-finding-study)
[redacted]
unpublished
BASF RegDoc# 1991/11248

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: Range-finding study, comparable to OECD 407 (1981)

Deviations: Comparable to OECD 407

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon), solid, yellow-beige
Purity: 94.1 %, isomer reduced
Batch No.: P.5314
Test substance No.: 88/174
Test animals: Beagle dogs

High purity (95 %) chloridazon was administered to two male and two female Beagle dogs per dose level over a period of 4 weeks via the feed. The dose levels were 0; 900; 2,700; 5000 and 10000 ppm.
Each dog was offered 700 g of food each day. The food consisted of a paste, made up from 350 g of solid food (with or without test substance) and 350 g of water.
The animals were examined each day for clinical symptoms and mortalities. Body weight was recorded at weekly intervals, while food intake was determined daily. Clinical-chemical and hematological investigations as well as urinalysis were performed before the start of the study and towards the end of the administration period. All animals were assessed gross-pathologically and subsequently subjected to a comprehensive histopathological examination.

Findings:
The stability of the test substance was demonstrated. The stability, homogeneity and the correctness of the concentration of the dog food were analytically verified.

Table B.6.3-4: Test substance intake

Dietary dose level (ppm)	Test substance intake males in mg/kg bw
900	30.7
2700	81.9
5000	112.6
10000	*

* Due to the severely reduced food consumption and premature sacrifice no meaningful data were obtained

Due to the poor general state of health of the animals of the high (10000 ppm) dose group, they were sacrificed prematurely on day 7. Most likely this is attributable to the severely reduced food consumption, which sometimes resulted in complete rejection of the food.
Reduced food consumption was also observed in the 5000 ppm group animals, see table below.

Table B.6.3-5: Relative food consumption, calculated in relation to the food provided each day in percent

Dose level (ppm)	Male	Females
0	100	91
900	100	100
2700	92	88
5000	70	53
10000	8*	8*

* sacrificed on day 7

The numerically slightly lower food consumption of the 2700 ppm animals is not considered to be test substance related as their body weight development was equal or better than in controls.
Clinical signs of toxicity consisted of vomiting in 10000 ppm and 5000 ppm groups.

Table B.6.3-6: Body weight change in kg

Dose level	Males	Females
0	- 0.1	- 0.2
900	+ 0.3	+ 0.3
2700	+ 0.1	+ 0.1
5000	- 0.9	- 1.4
10000	- 1.6*	- 1.5*

* sacrificed on day 7

The high dose (10000 ppm) animals were sacrificed on day 7, the findings of this group are listed below. The findings may have been related to the administration of chloridazon, however, due to the animals' state of health a definitive assessment is not possible.
One male and one female dog showed moderate cachexia. Pathological examinations in this group demonstrated reduced relative kidney weight in one male. Further observations consisted of minimal to slight vacuolisation of distal renal tubules in the females and hepatocellular vacuolisation in one male and one female.
In the 5000 ppm group the following test substance related findings were obtained:

Hematology:
- Decreased erythrocytes, hemoglobin and hematocrit in one female.
Clinical chemistry:
- Increased cholesterol in both sexes.
- Increased triglycerides in one male.
- Decreased glucose and creatinine in one female.
Pathology:
- Reduced absolute kidney and adrenal weights in one male and one female.
- Minimal to slight vacuolisation of distal renal tubules in females.
- Minimal to slight diffuse or focal hepatocellular vacuolisation in females.
- Hepatocellular vacuolisation in one male.
There were no test substance related findings in the 2700 ppm and 900 ppm groups.

Conclusion:
The NOAEL for male and female beagle dogs under the chosen test conditions was 2700 ppm (equivalent to approximately 81,9 mg/kg bw).

B.6.3.2 Oral administration (90-day studies)

B.6.3.2.1 Rat

Report: Hunter B. et al., 1975
Toxicity of pyrazon to rats. Dietary administration for 13 weeks (final report 0 - 19 weeks)
[redacted] unpublished
BASF RegDoc# 1975/028

GLP: No, studies were conducted prior to the implementation of GLP but are scientifically valid

Guideline: in accordance with OECD 408 (1981)

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Pyrazon (chloridazon), powder, sandy colored
Purity: not indicated
Batch No.: 942E 679
Test animals: Sprague-Dawley rats

Chloridazon was administered to Sprague-Dawley rats at dietary dose levels of 0; 300; 1200; 4800 and 19200 ppm over a period of 13 weeks. The 19200 ppm dose level was reduced to 9600 ppm after the 5th week of administration. The controls and high dose groups consisted of 20 males and 20 females each, whereas the other groups consisted of 15 males and 15 females each. At the end of the administration period all surviving rats, except 5 males and 5 females of the control and 4800 ppm group each, were killed. The remaining animals were maintained on a test substance free diet for a further 6 weeks to study recovery.
The animals were observed for clinical symptoms and mortalities. Body weight and food intake was measured weekly. Water consumption was assessed by regular inspection of the water bottles and was measured during weeks 5 and 13. Ophthalmoscopy was performed before the start of the study and during weeks 4 and 8 on all male and female control rats and high dose rats.
Hematological investigations were performed during weeks 0, 4, 8 and 11 on 10 males and 10 females from the control and high dose groups. During week 18 (recovery) investigations were performed on 5 males and 5 females of control and 4800 ppm dose groups.
On week 4 hematological investigations were performed using 10 females each of the 1200 ppm and 4800 ppm groups.
On weeks 8 and 11 hematological investigations were performed using 10 males and 10 females each of the 1200 ppm and 4800 ppm groups.
In addition, total white blood cell count was performed at week 4 using 10 males of the 4800 ppm group.
Clinical-chemical investigations were performed during weeks 4 and 11 on 5 males and 5 females from the control and high dose groups. During week 18 (recovery) investigations were performed on 5 males and 5 females of control and 4800 ppm dose groups.
On week 4 clinical-chemical investigations were performed using 5 males and 5 females of the 1200 ppm group and 5 females from the 4800 ppm group.
On week 11 clinical-chemical investigations were performed using 5 males and 5 females each of the 1200 ppm and 4800 ppm groups.
At week 12 urea was determined in 5 males and 5 females from the 300 ppm group.
Urinalysis was performed during weeks 4, 8 and 11 using 5 males and 5 females from the control and high dose groups. During week 18 (recovery) investigations were performed on 5 males and 5 females of control and 4800 ppm dose groups.
All animals were subjected to a gross macroscopic investigation. The weights of the adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thyroid and uterus were determined.
Histopathology was confined to:
- rats that died,

- 8 male and 8 female control animals, 10 females from the 4800 ppm group and all surviving rats from the high dose group,
- any tissues showing test substance related effects.

Findings:
The stability of the test substance and the stability of the dietary preparations were demonstrated in the long term rat and mouse studies, in which the same batch was used.

Table B.6.3-7: Test substance intake

Dietary dose level (ppm)	Test substance intake Males in mg/kg bw	Test substance intake Females in mg/kg bw
300	20.7	23.5
1200	83.0	84.8
4800	333.6	399.8
9600 (week 6 – 13)	716.0	686.4
19200 (week 1 – 5)	2326.9	1951.2

At the high dose level (19200 / 9600 ppm) 6 males and 17 females were sacrificed prematurely because of retardation of growth (loss of body weight), emaciation or loss of use of hind limbs. In addition, alopecia was noted in many animals of the high dose group.
No test substance related mortality or clinical signs of toxicity occurred in the study at the other dose levels.
Food consumption was statistically significantly reduced in the high and 4800 ppm dose levels (see table below).

Table B.6.3-8: Total food consumption (in gram over the indicated time period)

Dose group (ppm) / treatment time	0	300	1200	4800	19200 / 9600
Males week 1 – 5	870	907	875	845	626***
Females week 1 – 5	678	699	634	605*	508***
Males week 6 – 13	1368	1266	1399	1305	1194*
Females week 6 – 13	1113	1096	1042	994	744***

* P < 0.05; *** P < 0.001

Body weight gain was statistically significantly reduced in the high and 4800 ppm dose levels (see table below).

Table B.6.3-9: Body weight gain (in gram, over the indicated time period)

Dose group (ppm) / treatment time	0	300	1200	4800	19200 / 9600
Males week 1 – 5	263	273	262	229**	49***
Females week 1 – 5	133	137	121	91***	40***
Males week 6 – 13	129	148	149	136	208***
Females week 6 – 13	77	83	81	58**	31*** (#)

** P < 0.01; *** P < 0.001, # data from three surviving females

Water consumption in female high dose animals, when related to food intake, was observed to be increased. However, the absolute quantities of drinking water used were quite comparable amongst all groups.
Erythrocyte counts and hemoglobin were consistently lower in high dose females.

Table B.6.3-10: Erythrocytes and hemoglobin in females

Dose group (ppm) / treatment time	0	300	1200	4800	19200 / 9600
Erythrocytes (10^6/mL)					
Week 4	6.8	-	7.2**	7.3***	6.3***
Week 8	7.4	-	6.9**	7.0**	7.0**
Week 11	7.1	-	7.1	6.9	6.5**
Hemoglobin (g %)					
Week 4	14.8	-	14.9	14.8	12.7***
Week 8	15.2	-	14.2***	14.7*	14.6**
Week 11	15.1	-	14.7	14.7	13.5***

** P < 0.01; *** P < 0.001, - : not determined

There is no clear effect on red blood cell parameters for the 1200 and 4800 ppm females. For erythrocytes values are sometimes higher, sometimes lower than controls. Concerning hemoglobin there is no clear dose response relationship. For both parameters the intergroup variability is relatively high.
Clinical chemical investigations demonstrated several altered parameters for the high dose group;
Week 4: increased alanine aminotransferase, bilirubin, sodium, potassium and cholesterol.
Week 8: increased alkaline phosphatase (females) and alanine aminotransferase.
These results suggest an impaired liver function.
There were no effects on urinalysis and ophthalmoscopy.
Organ weights determination revealed an increase in absolute and relative liver weight as shown in the table below.

Table B.6.3-11: Liver weights

Dose group (ppm)	Absolute weight (g)		Relative weight	
	Males	Females	Males	Females
0	25.3	12.9	483	392
300	28.1*	13.6	498	395
1200	27.2	14.6*	511	447**
4800	27.0	16.2***	549**	585***
19200 / 9600	26.1	16.6***	711***	753***

* P < 0.05, ** P < 0.01; *** P < 0.001

Thus, relative liver weights were increased in males and females of the 4800 and high dose groups, as well as in females of the 1200 ppm group.
Histopathological investigations revealed a minimal enlargement of centrilobular hepatocytes and a decrease in glycogen content in periportal hepatocytes in animals of the 4800 ppm and high dose groups.
The occurrence of narrowing muscle fibre in high dose animals was considered to be related to the emaciated condition of these animals.

Conclusion:
The effects in high dose group (19200 / 9600 ppm) are partly related to the test substance, partly to the general poor state of health of the animals. At 4800 ppm toxicity is characterised by effect on food consumption, body weight. Target organ was the liver (increased weights and histopathological changes). The 1200 ppm group females showed increased liver weights, however, without changes in clinical chemistry or histopathology. These changes may be adaptive, rather than of toxicological significance.

The NOAEL in this study was 300 ppm (20.7 mg/kg bw in males and 23.5 mg/kg bw in females).

B.6.3.2.2 Mouse

Report: Schilling K., Hildebrand B., 1990
Study on the oral toxicity of Reg. No. 13 033 in B6C3F1 mice. Administration via the diet over 3 months
[redacted]
unpublished
BASF RegDoc# 1990/0568

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: EPA 82-1, OECD 408, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon)
Batch No.: P 5314
Purity: 94.1 %
Test substance No.: 88/174
Test animals: B6C3F1 mice

Chloridazon was administered to 10 male and 10 female B6C3F1 mice per group at dietary dose levels of 0; 300; 1500 and 7500 ppm over a period of 3 months.
The animals were daily observed for clinical symptoms and mortalities. Each week the animals were subjected to an additional detailed clinical examination. Body weight and food consumption was measured weekly. Clinical-chemical and hematological examinations were performed on all animals at the end of the administration period.
All animals were subjected to a full macroscopic and histopathological examination.

Findings:
The stability of the test substance was proven after termination of all studies.
The stability and homogeneity of the test substance in dietary preparations was proven. The correctness of the concentrations of the dietary preparation was analytically verified.

Table B.6.3-12: Test substance intake

Dietary dose level (ppm)	Test substance intake Males in mg/kg bw	Test substance intake Females in mg/kg bw
300	65	91
1500	319	467
7500	1756	2316

There were no mortalities or signs of clinical toxicity in any of the groups.
At the high dose level food consumption was reduced by approximately 7 % in both sexes. Body weight development was reduced in high dose males and females as well as in 1500 ppm males. Body weight in the high dose group was 18 % lower in males and 8 % in females. In males of the 1500 ppm group body weight was 12 % lower than controls.

Table B.6.3-13: Body weight and food consumption

Dose level (ppm)	Body weight (g)		Food consumption (g)	
	Males	Females	Males	Females
0	36.7	28.7	6.0	7.3
300	34.9	29.5	6.4	7.5
1500	32.4**	28.4	6.0	7.4
7500	30.2**	26.5	5.6	6.8

** P < 0.01

Clinical-chemical examinations revealed reduced triglycerides and cholesterol values in high dose and 1500 ppm males.

Table B.6.3-14: Triglyceride and cholesterol values in male mice

Dose level (ppm)	Triglycerides (mmol/L)	Cholesterol (mmol/L)
0	1.90	3.63
300	1.54*	3.36
1500	1.22**	3.15**
7500	0.86**	2.53**

* P < 0.05; ** P < 0.01

The reduction in triglycerides and cholesterol is likely to be related to the reduction in body weight. The reduction at 300 ppm for the males is not considered to be test substance related because 1) it is only marginal, 2) it occurs in males only, and 3) there is no indication for an effect on lipid metabolism at this dose level and body weights were not affected.
There were no test substance related findings in the hematological examinations.
Organ weight determinations revealed an increase in absolute and relative liver weight in high dose females. Relative liver weights were increased in high dose and 1500 ppm group males.

Table B.6.3-15: Absolute and relative liver weights

Dose level (ppm)	Absolute liver weight (gram)		Relative liver weight	
	Males	Females	Males	Females
0	1.149	1.074	3.585	4.343
300	1.129	1.122	3.767	4.515
1500	1.17	1.08	4.152**	4.567
7500	1.227	1.175*	4.782**	5.301**

* P < 0.05; ** P < 0.01

The observed statistically significantly increased relative kidney and adrenal weights in males were considered to be related to the reduction in body weight in these animals and not a direct test substance related effect.
Gross and histopathological investigations did not reveal any test substance related changes.

Conclusion:
The NOAEL in this study was 300 ppm for male mice (65 mg/kg bw) and 1500 ppm for female mice (467 mg/kg bw).

B.6.3.2.3 Dog

Report: Leuschner F. et al., 1975
Oral toxicity of pyrazon, Reg.-Nr. 13 033, techn. (88.7 %) - called for short 'Pyrazon' - in Beagle dogs (repeated dosage for 3 months)
[redacted]
unpublished
BASF RegDoc# 1975/029

GLP: No, studies were conducted prior to the implementation of GLP, but are scientifically valid; a GLP compliance statement is provided concerning the integrity of the study

Guideline: Not mentioned, study is similar to OECD 409 (1981)

Deviations: Age of the dogs 9-10 months

Acceptability: The study is considered to be acceptable as supportive.

Material and Methods:
Test material: Test substance was identified as follows:
Pyrazon (Reg. No. 13 033; chloridazon)
Purity: 88.7 %
Test animals: Beagle dogs

Chloridazon was administered to four male and four female Beagle dogs at dietary dose levels of 0; 100; 300; 900 and 10000 ppm over a period of 3 months.
The animals were daily observed for clinical symptoms and mortalities. Body weight was measured weekly. The animals were subjected to a full neurological examination, including pupillary light reflex, corneal reflex, patella reflex, flexor and extensor reflex as well as postural reflex.
Food intake was determined daily. Drinking water consumption was also noted. Body weight was determined once a week.
Ophthalmoscopy and an auditory test were performed at the end of the administration period. Clinical-chemical and hematological examinations as well as urinalysis were performed on all animals on weeks 6 and 13 week of the administration period. Clinical-chemical analysis consisted of the following parameters: Alanine aminotransferase, aspartate aminotransferase, glucose, blood urea nitrogen, total protein, albumine, globuline, total bilirubin, alkaline phosphatase, sodium, potassium, calcium, chloride, uric acid, liver function (bromsulphatein) test. In addition an electrocardiogram was made before and 2 hours after the administration.

All animals were subjected to a full macroscopic pathological examination. Weights of selected organs were determined. Histopathological investigations were performed.

Findings:
Test substance intake was calculated based on the average intake of test substance of weeks 1, 6 and 13.

Table B.6.3-16: Approximate test substance intake

Dietary dose level (ppm)	Test substance intake in males and females (mg/kg bw)
100	3.1
300	10.1
900	28.8
10000	326

There were no mortalities or signs of clinical toxicity (including electro-cardiogram, auditory test and neurological examinations) in any of the groups.
In the high dose group the following test substance related effects were observed:
Food consumption was slower, however, the amounts of food consumed were comparable to the controls. Water consumption was not affected.
Body weight development was reduced, resulting in a slightly lower average body weight at the end of the treatment period (10000 ppm: 10.1 kg, controls: 10.6 kg).
Clinical-chemistry demonstrated slightly lower values for total serum protein, albumine and globuline.
There were no test substance related findings in the hematological examinations and urinalysis.
Organ weight determinations revealed a decrease in absolute and relative testes, prostate, uterus and thyroid weight. The extent of the organ weight reductions were in the range of the reduction in body weight.
Gross and histopathological investigations did not reveal any test substance related changes.

Conclusion:
The NOAEL in this study was 900 ppm (i.e. 28.8 mg/kg bw) for male and female dogs.

Report: Hellwig J., Hildebrand B., 1992
Report on the study of the toxicity of Reg. No. 13 033; 95 % in Beagle dogs. Administration via the diet over 3 months
[redacted]
unpublished
BASF RegDoc# 1992/11648

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: OECD 409, EPA 82-1

Deviations: None, however no toxic dose, study followed-up by study BASF RegDoc #1993/10823

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon), solid brown
Batch No.: N 143
Purity: 94.1 %
Test substance No.: 88/174-1
Test animals: Beagle dogs

Chloridazon was administered to each 6 male and 6 female Beagle dogs per test group over a period of 3 months via their diet. The dose levels were 0; 300; 1000 and 3000 ppm. Feed consumption of the animals was determined daily and their body weight once a week; the dogs state of health was checked every day.
Clinicochemical and hematological examinations as well as urinalyses were carried out once before and two times (after approximately 5 and 13 weeks of treatment) during the administration period.
Ophthalmological examinations were carried out before the beginning of the study and towards the end of the administration period.
All animals were assessed gross-pathologically and subsequently subjected to a comprehensive histopathological examination.

Findings:
The stability of the test substance was demonstrated. The stability, homogeneity and the correctness of the concentration of the dog food was analytically verified. For the high dose group single deviations from target were 10.1 % at the most. This is considered not to have affected the study.

Table B.6.3-17: Test substance intake

Dietary dose level (ppm)	Test substance intake in males and females in mg/kg bw
300	10
1000	32
3000	97

There were no test substance related findings at any dose level.

Conclusion:
In conclusion, the 3-month administration of chloridazon was tolerated by the animals (males and females) without any changes that could be causally related to the test substance administered.

The NOAEL in this study was 3000 ppm (i.e. 97 mg/kg bw) for male and female dogs.
As there were no toxic effects in this study, a subsequent 3-months study using higher doses (4000 and 5000 ppm) was performed [see 1993/10823 Mellert W. 1993].

Report: Mellert W., 1993
Supplementary study of the toxicity with Reg. No. 13 033; 95 % in Beagle dogs. Administration in the diet for 3 months
[redacted]

unpublished
BASF RegDoc# 1993/10823

GLP: Yes (laboratory certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 31 80, 6500 Mainz 1)

Guideline: OECD 409, EPA 82-1, EEC 87/302, JMAFF

Deviations: No dose without effect, complementary study to study BASF RegDoc# 1992/11648.

Acceptability: The study is considered to be acceptable in conjunction with study BASF RegDoc# 1992/11648.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon), solid brown
Batch No.: N 143
Purity: 93.4 %
Test substance No.: 88/174-1
Test animals: Beagle dogs

Chloridazon was administered to each 6 male and 6 female Beagle dogs per test group over a period of 3 month via their diet. The dose levels were 0; 4000 and 5000 ppm. This study was conducted as a supplementary study, because in a previous 3-month dog study [see 1992/11648, Hellwig J., Hildebrand B., 1992] no toxic effects were observed up to a dose level of 3000 ppm.
Feed consumption of the animals was determined daily and their body weight once a week; the dogs' state of health was checked every day.
Clinicochemical and hematological examinations as well as urinalyses were carried out once before and two times (after approximately 5 and 13 weeks of treatment) during the administration period.
Ophthalmological examinations were carried out before the beginning of the study and towards the end of the administration period.
All animals were assessed gross-pathologically and subsequently subjected to a comprehensive histopathological examination.

Findings:
The stability of the test substance was demonstrated. The stability, homogeneity and the correctness of the concentration of the dog food were analytically verified.

Table B.6.3-18: Test substance intake

Dietary dose level (ppm)	Test substance intake in males and females in mg/kg bw
4000	133
5000	168

There were no signs of clinical toxicity or mortalities in any dose group.

Food consumption and body weight change were slightly impaired in female dogs treated with 4000 and 5000 ppm.

Table B.6.3-19: Food consumption and bodyweight change in female dogs

Dose level (ppm)	0	4000	5000
Food consumption (% of controls)	100 %	96 %	96 %
Body weight change (kg, after 3 months)	1.7	1.3	1.2

There were no changes in the ophthalmological, hematological, clinical-chemical or urinalysis investigations.
Liver weight in male dogs treated with 4000 and 5000 ppm were found to be increased (see table below). In addition, relative kidney weight in high dose males was increased (controls: 0.428; 5000 ppm: 0.469).

Table B.6.3-20: Liver weight changes

Dose level (ppm)	0	4000	5000
Absolute liver weight (g)	344.632	391.752*	368.293
Relative liver weight	2.893	3.326**	3.226**

* P < 0.05; ** P < 0.01

There were no macroscopic test substance related changes.
Histopathological investigations demonstrated cellular vacuolisation of the distal renal tubules of female dogs treated with 4000 and 5000 ppm.

Conclusion:
The 3-month administration of high doses of chloridazon to dogs resulted in slight effects on food consumption and body weight. Target organs were the kidneys and the liver.
The NOAEL in this study was below 4000 ppm (133 mg/kg bw) for male and female dogs.
The combined evaluation of the two 3-month dog studies [see 1992/11648, Hellwig J., Hildebrand B., 1992] and [see 1993/10823, Mellert W., 1993] demonstrates an overall low observed adverse effect level of 4000 ppm (133 mg/kg bw) and an overall NOAEL of 3000 ppm (97 mg/kg bw).

B.6.3.3 Oral administration (1-year studies, dog)

Report: Mellert W. et al., 1993(a)
Chronic toxicity study with Reg. No. 13 033; 95 % in Beagle dogs.
Administration in the diet for 12 months
[redacted]
unpublished
BASF RegDoc# 1993/10815

GLP: Yes (laboratory certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 31 80, 6500 Mainz 1)

Guideline: OECD 452, EPA 83-1, EEC 87/302, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon), solid, brown
Purity: 94.1 %
Batch No.: N 143
Test substance No.: 88/174-1
Test animals: Beagle dogs

Chloridazon was administered for 12 months to 6 male and 6 female Beagle dogs per group at dietary dose levels of 0; 400; 1200 and 3600 ppm. Feed consumption was determined daily and body weight was determined once a week. The animals state of health was checked each day.
Clinical-chemical and hematological examinations and urinalysis were carried out once before and after approximately 3, 6 and 12 months of administration. Ophthalmological examinations were carried out before the start of the study and towards the end of the administration period.
All animals were assessed gross-pathologically and subsequently subjected to a complete histopathological examination.

Findings:
The stability of the test substance over the study period and the stability of the test substance in the food was analytically confirmed. The homogeneity and the correctness of the dietary concentrations were also analytically confirmed.

Table B.6.3-21: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw)
400	11
1200	33
3600	99

There were no mortalities, clinical signs of toxicity or adverse effects on body weight development noted in any of the treatment groups that could be related to the administration of the test substance.
There were no test substance related changes in hematology, clinical chemistry and urinalysis at any dose level. Ophthalmoscopy did not demonstrate test substance related effects.
There were no test substance related effects on body weight or gross-pathological findings at any dose level.
Histopathology showed a slightly increased incidence and severity of haemosiderin storage in the spleens of high dose males and females.

Table B.6.3-22: Haemosiderin storage in the spleen

	Males				Females			
dose level	0	400	1200	3600	0	400	1200	3600
incidence	4	2	2	6	4	1	2	6
grade 1	4	1	1	3	4	1	2	3
grade 2	0	1	1	2	0	0	0	2
grade 3	0	0	0	1	0	0	0	1

In the liver of high dose males an increased incidence but not severity of haemosiderin storage was observed in the Kupffer cells.

Table B.6.3-23: Haemosiderin storage in the liver

	Males				Females			
dose level	0	400	1200	3600	0	400	1200	3600
incidence	1	2	2	4	4	3	5	1

As can be seen from the tables, the increase was numerically slight and concerning the liver not consistent in males and females. Moreover, no corresponding findings were obtained in hematological and clinical chemical investigations. Therefore, the finding is not considered to be of toxicological significance.
In a further 12 month dog study [see 1993/10824, Mellert W. et al., 1993d] with higher dose levels, this finding was not observed and can therefore be assessed as incidental.

Discussion:
Body weight changes were seen in females in dose groups 1200 ppm and 3600 ppm over the course of treatment, which became significant during individual weeks in the second half of the study.

Conclusion:
The NOAEL in this study was 400 ppm, which is equivalent to 11 mg/kg bw.

Report: Mellert W. et al., 1993(d)
Supplementary chronic toxicity study with Reg. No. 13 033; 95 % in Beagle dogs. Administration in the diet for 12 months
[redacted]
unpublished
BASF RegDoc# 1993/10824

GLP: Yes (laboratory certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 31 80, 6500 Mainz 1)

Guideline: OECD 452, EPA 83-1, EEC 87/302, JMAFF

Deviations: Complementary study, 2 dose groups with toxic effects, 1 control group

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Test substance was identified as follows:

Reg. No. 13 033 (chloridazon), solid, brown
Purity: 93.75 %
Batch No.: N 143
Test substance No.: 88/174-1

Test animals: Beagle dogs

Chloridazon was administered for 12 months to 6 male and 6 female Beagle dogs per group at dietary dose levels of 0, 6000 and 8000 ppm. Feed consumption was determined daily and body weight was determined once a week. The animals' state of health was checked each day. Clinical-chemical, hematological examinations and urinalysis were carried out once before and after approximately 3, 6 and 12 months of administration. Ophthalmological examinations were carried out before the start of the study and towards the end of the administration period.
All animals were assessed gross-pathologically and subsequently subjected to a complete histopathological examination.

Findings:

The stability of the test substance over the study period was proven by re-analysis after termination of all of the studies performed with this batch.
The stability of the test substance in the food was analytically confirmed. The homogeneity and the correctness of the dietary concentrations were also analytically confirmed.

Table B.6.3-24: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw)
6000	186
8000	241

There were no mortalities or clinical signs of toxicity in any of the treatment groups that could be related to the administration of the test substance.
Food consumption was slightly impaired in both sexes at both dose levels. In males, however, this effect occurred only during the first month of the study at both dose levels. Over the entire study food consumption was 97 % and 93 % of control values in males and females at 8000 ppm. At 6000 ppm these values were 100 % and 94 % in males and females respectively.
Body weight in 8000 ppm males was impaired during the first month of the study, thereafter no test substance related impairment was observed.
Body weight in 6000 and 8000 ppm females was impaired during the whole study period. At the end of the administration period body weights were 16 % and 12 % lower than controls at 6000 and 8000 ppm respectively.
Body weight gain (difference in body weight between study day 0 and 364) is shown in Table B.6.3-25.

Table B.6.3-25: Body weight gain in kg

Dose level (ppm)	Males	Females
0	1.9	2.9
6000	0.9	0.8
8000	1.8	1.2

The apparent lack of a dose response relationship is probably related to the close spacing of the dose levels.

There were no test substance related changes in hematology and urinalysis at any dose level.
Ophthalmoscopy did not demonstrate test substance related effects.
Clinical chemistry demonstrated an increase in inorganic phosphate in both sexes at both dose levels and a decrease in total bilirubin in 8000 ppm males.
There were no test substance related effects on body weight or gross-pathological findings at any dose level.
Histopathological examinations revealed:
Vacuolisation of distal renal tubules in females at 6000 and 8000 ppm.
Lymphofollicular hyperplasia of gastric mucosa in males and females at 6000 and 8000 ppm. This finding may have been due to a slight gastric irritation caused by the test substance.

Discussion:
The incidence of both renal and gastric changes and partly the degree of severity represent treatment related effect, likely to be caused by slight gastric irritation in both sexes and by minimal nephrotoxicity in females.

Conclusion:
Both doses of 6000 and 8000 ppm investigated in this study resulted in substance related observations. A NOAEL could not be established in this study.

The NOAEL had been established in a previous study [see 1993/10815, Mellert W. et al., 1993a] at 400 ppm (11 mg/kg bw).

B.6.3.4 Other routes

Report: Kirsch P., Hildebrand B., 1992
Study of the dermal toxicity of Reg. No. 13 033; 95 % in White rabbits. Application to the intact skin over 3 weeks
[redacted]
unpublished BASF
RegDoc# 1992/10413

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: EPA 82-2, OECD 410

Deviations: None

Acceptability: Limit test, one dose group of 1000 mg/kg bw, one control group.
The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon), powder, brown
Purity: 93.36 %, isomer reduced

Batch No.: N 143
Test substance No.: 88/174-1
Test animals: New Zealand White rabbits

Chloridazon was applied to the clipped intact dorsal skin of five male and five female New Zealand White rabbits per dose level for a period of six hours per day for three weeks using a semiocclusive dressing. The concentrations were 0 (0.5 % aqueous suspension of Tylose CB 30.000, i.e. sodium carboxymethyl cellulose) and 1000 mg/kg bw.
The animals were examined twice daily for clinical symptoms and mortalities. Local irritation was recorded daily, about 30 min after removal of the dressing.
Clinical-chemical and hematological investigations were performed at the end of the administration period. All animals were assessed by gross pathology, followed by a histopathological examination.

Findings:
The stability of the test substance was demonstrated. The stability, homogeneity and the correctness of the concentration of the test substance preparation were analytically verified. The analytical verified concentrations of the preparation were 118.6 – 124 % of the nominal concentrations.
There were no mortalities or clinical signs of toxicity in the treatment group.
Body weight and food consumption were not affected.
Clinical-chemical and hematological examinations did not reveal test substance related changes.
Gross macroscopic observations and histopathological examinations did not show any treatment related effects.
Absolute and relative adrenal weights were decreased in male rabbits. However, as there were no macroscopic or microscopic changes to the adrenals as well as no indication from any of the other parameters including clinical chemistry of a test substance related effect, the reduced adrenal weights were assessed not to be related to treatment.
There were no signs of skin irritation in any of the animals.

Conclusion:
The NOAEL for systemic toxicity was 1000 mg/kg bw.
Signs of local irritation were not observed.

B.6.4 Genotoxicity (Annex IIA 5.4)

Chloridazon was evaluated for possible mutagenic/genotoxic effects in vitro and in vivo. It was negative in 4 out of 5 reverse mutation assays in bacteria (Ames test). The only positive response was obtained in one of the oldest tests [see 1977/037 Oesch F. 1977] with the isomer rich quality at doses causing test substance precipitation. In this study precipitation was observed at very low doses (1,000 µg/plate) in contrast to the other studies. In addition, the effect observed was marginal (maximum 1.5 fold increase with and without metabolic activation). Such marginal increased mutation frequency taking also into account the negative results obtained with other batches suggests that an impurity or physical properties (precipitation) may have been responsible for this finding. This effect could not be reproduced in other batches of chloridazon either with isomer reduced or non-reduced samples using the same test strains and solvent (DMSO). In these studies the concentrations included the same dose range and were even above (up to 5,000 µg/plate) the dose causing a positive reaction and in some

experiments the top dose level did not even cause test substance precipitation. Thus it can be concluded that the present isomer reduced technical active ingredient is negative in the Ames test including *Salmonella* and *E. coli* tester strains. In a 6th reverse mutation assay reverse mutation assay in a special *E. coli* strain (*E. coli* K 12 (343/113)) chloridazon was also negative. Only under special in vitro conditions (pH 1, nitrosation) the chemical reaction product N-methyl-nitroso-aniline was positive in this test system.
Point mutations in mammalian cells were assessed in Chinese hamster ovary cells (HPRT assay). In this study no mutagenic effects were observed.
In the chromosome aberration test in vitro in human lymphocytes no increase of mutation frequency was noted.
DNA damage and repair was investigated in vitro in bacterial cells (recombination assay with *Bacillus subtilis*) in two experiments and in the UDS test in primary rat hepatocytes. In all 3 experiments there was no indication of DNA damage and repair caused by chloridazon.
In two in vivo studies NMRI mice, a micronucleus tests (single oral application) and a dominant lethal assay (gavage for five consecutive days), the absence of a genotoxic potential of chloridazon was confirmed.
The studies sufficiently cover all endpoints required for mutagenicity and genotoxicity testing. It is concluded that chloridazon has no mutagenic or genotoxic potential.

Table B.6.4-1: Genotoxicity testing results with chloridazon

Author/year/ laboratory	Test system	Strain/species	Test conditions	Result
[1989/0173; Engelhardt G., Hoffmann H. D.; 1989] BASF	Point mutation bacterial cells Ames test	*S. typhimurium* TA 1535, TA 100, TA 1537, TA 98 and E. coli WP2 uvrA	With S9 mix Without S9 mix Standard plate and preincubation assay	Negative
[1988/0208; Engelhardt G., Hoffmann H. D.; 1988b] BASF	Point mutation bacterial cells Ames test	*S. typhimurium* TA 1535, TA 100, TA 1537, TA 98	With S9 mix Without S9 mix Standard plate and preincubation assay	Negative
[1988/0207; Engelhardt G., Hoffmann H. D.; 1988a] BASF	Point mutation bacterial cells Ames test	*S. typhimurium* TA 1535, TA 100, TA 1537, TA 98	With S9 mix Without S9 mix Standard plate and preincubation assay	Negative
[1976/012; Shirasu Y. et al.; 1976] IET / Japan	Point mutation bacterial cells Ames test	*S. typhimurium* TA 1535, TA 100, TA 1537, TA 98, TA 1538 and E. coli WP2 hcr	With S9 mix Without S9 mix Standard plate assay	Negative
[1977/037; Oesch F.; 1977] University of Mainz	Point mutation bacterial cells Ames test	*S. typhimurium* TA 100, TA 1537, TA 98	With S9 mix Without S9 mix Standard plate assay	Weakly positive factor<1.5
[1977/10242; Egert G. et al.; 1977] Publication	Point mutation bacterial cells	Publication: no details on study design *E. coli* K 12 (343/113)	With metabolic activation (mouse liver microsomes, no information on type of enzyme induction) and presumably also without activation	Negative

Author/year/ laboratory	Test system	Strain/species	Test conditions	Result
[1990/0292; Jaeckh R., Hoffmann H. D.; 1990] BASF	Point mutation mammalian cells	Chinese Hamster ovary cells (HPRT locus)	With S9 mix Without S9 mix	Negative
[1988/0389; Engelhardt G., Hoffmann H. D.; 1988c] BASF	Chromosome aberration mammalian cells	Human lymphocytes	With S9 mix Without S9 mix	Negative
[1989/0192; Jagannath D. R.; 1989] Hazleton / USA	DNA damage Rec assay	*Bac. Subtilis* M 45 and H 17	With S9 mix Without S9 mix	Negative
[1976/012; Shirasu Y. et al.; 1976] IET / Japan	DNA damage Rec assay	*Bac. Subtilis* M 45 and H 17	Without metabolic activation	Negative
[1986/257; Cifone M. A., Myhr B. C.; 1986] Hazleton / USA	Unscheduled DNA synthesis in vitro	rat primary hepatocytes	-	Negative
[1987/0424; Engelhardt G., Gelbke H.-P.; 1987] [redacted]	In vivo micronucleus test	NMRI mice	Oral dose (single application): 0, 150, 300, 600 mg/kg bw	Negative
[1975/008; Anonymous; 1975] [redacted] [1981/10088; Merkle J.; 1981] [redacted]	Dominant lethal test	NMRI mice	Oral dose (5 applications): 570 mg/kg bw	Negative

B.6.4.1 In vitro testing

B.6.4.1.1 Gene mutation in bacterial cells

Chloridazon was negative in 4 out of 5 reverse mutation assays in bacteria (Ames test). The only positive response was obtained in one of the oldest tests [see 1977/037 Oesch F. 1977] at doses causing test substance precipitation. In this study precipitation was observed at very low doses (1,000 µg/plate) in contrast to the other studies. In addition, the effect observed there was marginal (maximum 1.5 fold increase with and without metabolic activation). Such marginal increased mutation frequency taking also into account the negative results obtained with other batches suggests that an impurity may have been responsible for this finding. This effect could not be reproduced with other batches of chloridazon either with isomer reduced (3 studies) or non-reduced samples (1 study) using the same test strains and solvent (DMSO). It was observed that the "positive" study performed with the non isomer reduced material, precipitation was noted at 1000 µg/plate and higher. In contrast to the isomer reduced mate-

rial complete solubility was obtained up to 5000 µg/plate. This finding also suggests a difference in the composition of the older non isomer reduced material compared to the new quality. Thus it can be concluded that the present technical active ingredient is negative in the Ames test including *Salmonella* and *E. coli* tester strains. In a 6th experiment in a special *E. coli* strain chloridazon was also negative. Only under special in vitro conditions (pH 1, nitrosation) the chemical reaction product N-methyl-nitroso-aniline was positive in this test system.

Report: Engelhardt G., Hoffmann H. D., 1989
Report on the study of Reg. No. 13 033; isomer reduced, (ZST test substance No.: 88/174) in the Ames Salmonella/mammalian microsome mutagenicity test and reverse mutation assay - *E. coli* WP2 uvrA (standard plate test and preincubation test)
BASF AG, Ludwigshafen/Rhein, Germany
unpublished
BASF RegDoc# 1989/0173

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: OECD 471, OECD 472

Deviations: For *E. coli* only strain WP2 uvrA was used

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon); isomer reduced, brown powder
Purity: 94.1 %
Batch No.: N 143
Test substance No.: 88/174
Test system: *Salmonella typhimurium* tester strains TA 1535, TA 100, TA 1537 and TA 98, *E. coli* strain WP2 uvrA

Chloridazon was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation (S-9 mix from male Sprague Dawley rats treated with Aroclor 1254). The following *Salmonella typhimurium* tester strains were used in this assay: TA 1535, TA 100, TA 1537 and TA 98. In addition the *E. coli* strain WP2 uvrA was also tested. The testing was performed according to the standard plate assay as well as preincubation assay.
The study consisted of 4 experiments:
1st experiment: the strains TA 1535, TA 100, TA 1537 and TA 98 were exposed at chloridazon doses ranging from 20 to 5,000 µg/plate in the standard plate test with and without metabolic activation.
2nd experiment: the strains TA 100 and 1537 were exposed to chloridazon doses ranging from 20 to 5000 µg/plate in the standard plate test with and without metabolic activation.
3rd experiment: the strains TA 1535, TA 100, TA 1537 and TA 98 were exposed to chloridazon doses from 20 to 5,000 µg/plate in the preincubation test with and without metabolic activation.

4th experiment: the strain *E. coli* WP2 uvrA was exposed to chloridazon doses ranging from 20 to 5,000 µg/plate in the standard plate test and preincubation test with and without metabolic activation.

Dimethylsulphoxide (DMSO) was used to solve the test substance and was used as solvent control. The positive controls (prepared in DMSO) were: 2-AA (2-aminoanthracene) with S9 mix and MNNG (N-methyl-N'-nitro-N-nitroso-guanidine), NPD (4-nitro-o-phenylendiamine) and AAC (9-aminoacridine chloride monohydrate), ENNG (N-ethyl-N-nitro-N-nitroso-guanidin) without S9 mix.

Findings:
Bacteriotoxicity or precipitation of test substance was not observed in any *Salmonella typhimurium* or *E. coli* strains with chloridazon in any assay. The test substance and DMSO did not induce two-fold increases in the number of revertant colonies at any dose level that would be criteria for a positive response. The mutagenic response of the positive controls indicates that the test system was able to detect mutagens.

Conclusion:
According to the results of the present study chloridazon was not mutagenic in the bacterial reverse mutation assay with *S. typhimurium* and *E. coli* under the experimental conditions chosen.

Report: Engelhardt G., Hoffmann H. D., 1988(b)
Report on the study of Reg. No. 13 033/chloridazon; isomer reduced (ZST test substance No.: 88/108) in the Ames test (standard plate test and preincubation test with *Salmonella typhimurium*)
BASF AG, Ludwigshafen/Rhein, Germany,
unpublished
BASF RegDoc# 1988/0208

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 31 60, 6500 Mainz)

Guideline: OECD 471

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 / chloridazon; isomer reduced
Purity: 94 %
Batch No.: 'Partie 5314'
Test substance No.: 88/108
Test system: *Salmonella typhimurium* tester strains TA 1535, TA 100, TA 1537 and TA 98

Chloridazon was tested for mutagenicity in the reverse mutation assay in bacteria with and without metabolic activation (S-9 mix from male Sprague Dawley rats treated with Aroclor