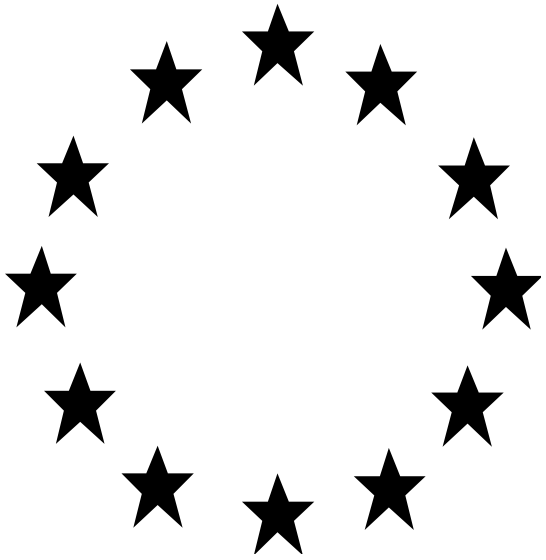

Draft Assessment Report (DAR)

- 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 2

July 2005

1254). The following *Salmonella typhimurium* tester strains were used in this assay: TA 1535, TA 100, TA 1537 and TA 98. The testing was performed according to the standard plate assay as well as preincubation assay.

The study consisted of 5 experiments:
1st experiment: the strains TA 98 and TA 100 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 strain TA 100 was exposed to chloridazon doses ranging from 100 to 7,500 µg/plate in the standard plate test with and without metabolic activation.
3rd experiment: the strains TA 1535 and TA 1537 were exposed to chloridazon doses from 20 to 5,000 µg/plate in the standard plate test with and without metabolic activation.
4th experiment: the strains TA 1535, TA 100, TA 1537 and TA 98 were exposed to chloridazon doses ranging from 20 to 5,000 µg/plate in the preincubation test with and without metabolic activation.
5th experiment: the strain TA 98 was exposed to chloridazon doses ranging from 500 to 5,000 µg/plate in the 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) without S9 mix.

Findings:
A weak bacteriotoxic effect was only observed in the strain TA 100 in the range of 500 - 5,000 µg/plate without and at 7,500 µg/plate with metabolic activation. Incomplete solubility of chloridazon was only observed at the 7,500 µg/plate dose level. The test substance and DMSO did not induce two-fold increases in the number of revertant colonies at any dose level that would be a 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* under the experimental conditions chosen.

Report: Engelhardt G., Hoffmann H. D., 1988(a)
Report on the study of chloridazon/Oxon, Italia; isomer free (ZST test substance No.: 88/107) in the Ames test (standard plate test and preincubation test with *Salmonella typhimurium*)
BASF AG, Ludwigshafen/Rhein, Germany,
unpublished
BASF RegDoc# 1988/0207

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:
Chloridazon/Oxon, Italia; isomer free
Purity: 94 – 97 %
Batch No.: Oxon Charge
Test substance No.: 88/107
Test system: *Salmonella typhimurium* tester strains TA 1535, TA 100, TA 1537 and TA 98

Chloridazon (supplied from Oxon/Italy) 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. The testing was performed according to the standard plate assay as well as preincubation assay.

The study consisted of 3 experiments:
1st experiment: the strains TA 98 and TA 100 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 1535 and 1537 were exposed to chloridazon doses ranging from 20 to 5,000 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.

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) without S9 mix.

Findings:
A weak bacteriotoxic effect was only observed in the strain TA 100 at doses $\geq$ 2,500 µg/plate. Complete solubility of chloridazon in DMSO was observed in all experiments. The test substance and DMSO did not induce two-fold increases in the number of revertant colonies at any dose level that would be a 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* under the experimental conditions chosen.

Report: Shirasu Y. et al., 1976
Mutagenicity testing on Pyramin in microbial systems
The Institute of Environmental Toxicology; Kodaira-shi, Tokyo 187; Japan
unpublished
BASF RegDoc# 1976/012

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

Guideline: Not indicated, however, the method used complies to a great extent to OECD 471 (1983)

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Chloridazon - former name pyrazon
Purity: 88.3 %
Batch No.: not mentioned
Test system: *Salmonella typhimurium* tester strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100

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 1254). The following *Salmonella typhimurium* tester strains were used in this assay: TA 1535, TA 1537, TA 1538, TA 98 and TA 100. In addition the *E. coli* strain WP2 hcr was tested. The testing was performed according to the standard plate assay.
The test was performed with the above mentioned strains for the standard plate assay, at doses ranging from 10 to 3,000 µg/plate without metabolic activation and from 10 to 1,000 µg/plate with metabolic activation. Dimethylsulphoxide (DMSO) was used to solve the test substance. The positive controls (prepared in DMSO) were: 2-AA (2-aminoanthracene) with and without S9 mix and AF-2, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, 2-nitrofluorene, 2-aminoanthracene without S9 mix.

Findings:
Bacteriotoxicity was not observed. Also no significant increase in positive reaction was observed in all strains treated with chloridazon. 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* under the experimental conditions chosen.

Report: Oesch F., 1977
Ames test for chloridazone
Dept. of Toxicology and Pharmacology, University of Mainz, Mainz, Germany,
unpublished
BASF RegDoc# 1977/037

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

Guideline: Not indicated, however, the method used complies to a great extent to OECD 471 (1983)

Deviations: Comparable to OECD 471

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Chloridazon
Purity: 84.6 %, technical grade
Batch No.: not indicated
Test substance No.: 77/356
Test system: *Salmonella typhimurium* tester strains TA 1537, TA 98 and TA 100

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 1254). The following *Salmonella typhimurium* tester strains were used in this assay: TA 1537, TA 98 and TA 100. The testing was performed according to the standard plate assay.
One or two independent tests depending on the strain used were performed in the presence or absence of metabolic activation. The doses ranged from 3 to 2,000 µg/plate in all tests. Dimethylsulphoxide (DMSO) was used to solve the test substance. The positive controls (prepared in DMSO) were: 2-AA (2-aminoanthracene), benzo(a)pyrene and 3-methylcholanthrene with S9 mix and MNNG (N-methyl-N'-nitro-N-nitroso-guanidine) and benzo(a)pyrene-4,5-oxide without S9 mix.

Findings:
No bacteriotoxicity was observed. A strong precipitation of chloridazon was observed at doses of 1,000 µg/plate and above. Chloridazon was negative in the strain TA 1537 with and without metabolic activation. A positive response was observed in the strain TA 100 with and without metabolic activation in two independent experiments. In TA 98 the positive response was observed in the presence of metabolic activation in two experiments and in the absence of metabolic activation it was only positive in one of the two experiments. Even at doses leading to strong precipitation of the test substance the increase in mutation frequency was small (1,5 fold). 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 weakly mutagenic in the bacterial reverse mutation assay with *S. typhimurium* under the experimental conditions chosen.

Report: Egert G. et al., 1977
Formation of promutagens from the herbicides pyrazon and chlorthiamid in the presence of nitrous acid
Mutation research, No. 46, 217-218
BASF RegDoc# 1977/10242
published

GLP: No, not subject of GLP regulations; study was conducted in a research institute at university

Guideline: Not applicable

Deviations: Publication, insufficient information on study design, conduct under artificial, not relevant conditions.

Acceptability: The study is considered to be not acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Chloridazon (pyrazon)
Purity: no information given
Batch No.: no information given
Test system: *E. coli* K-12 (343/113)

Pyrazon (chloridazon) has been examined for point mutation in bacterial cells (*E. coli* K-12 (343/113) in the presence and presumably also absence of metabolic activation (mouse liver microsomes, no information on type of enzyme induction). Doses were not mentioned in this brief publication. In addition the authors tested n-methyl-nitroso-aniline which has been obtained when 10^{-3} M chloridazon reacted in the presence of 10^{-3} M Na-thiocyanate and 10^{-1} M Na-nitrite at pH 1 with a yield of 6 %.

Findings:
Chloridazon was quoted to be negative under any testing conditions while n-methyl-nitroso-aniline was positive in the presence of metabolic activation in this test system.

Conclusion:
According to the results of the present study chloridazon was not mutagenic in a bacterial reverse mutation assay in *E. coli* K-12 (343/113) cells under the experimental conditions chosen. In vitro nitrosation at low pH led to a mutagenic compound in this test system.

B.6.4.1.2 Gene mutation in mammalian cells

Report: Jaeckh R., Hoffmann H. D., 1990
Report on a point mutation test carried out on CHO cells (HGPRT locus) with the test substance Reg. No. 13 033 (isomer reduced)
BASF AG, Ludwigshafen/Rhein, Germany,
unpublished
BASF RegDoc# 1990/0292

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

Guideline: OECD 476

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 (isomer reduced) / chloridazon
Purity: 94.1 %
Batch No.: N 143
Test substance No.: 88/174-1
Test system: Chinese hamster ovary (CHO-K1)

Chloridazon was examined for mutagenic activity (using the HPRT locus) by assaying for the induction of 6-thioguanine resistant mutants in Chinese hamster ovary (CHO-K1) cells after in vitro treatment. The test was performed with and without metabolic activation (S-9 mix from the liver of arochlor 1254 induced male Sprague-Dawley rats). The test concentrations were chosen based on the results of a preliminary cytotoxicity test. Two independent experiments were carried out. Concentrations of the test substance ranged from 0.05 to 5 mg/mL without S9 mix and from 0.05 to 5 mg/mL with S9 mix. The solvent 2 % DMSO was used as negative control while ethylmethanesulphonate (EMS) and 3-methylcholanthrene (MCA) were used as positive controls with or without S9-mix activation.

Findings:
Precipitation upon dilution of stock solution with medium was noted at a concentration of 5 mg/mL. In the first experiment cytotoxicity (no cell growth) was observed at 5 mg/mL with and without metabolic activation. In the second experiment cytotoxicity (reduced cloning efficiency) was observed at 1 mg/mL in the presence of metabolic activation and at 5 mg/mL (no cell growth) in the presence and absence of metabolic activation.
No biologically significant increases in mutant frequency were observed with and without metabolic activation. The positive controls demonstrated that the system was able to detect known mutagens.

Conclusion:
According to the results of the present study chloridazon was not mutagenic in the in vitro mammalian cell (CHO / HPRT) test under the experimental conditions chosen.

B.6.4.1.3 In vitro cytogenetic tests

Report: Engelhardt G., Hoffmann H. D., 1988(c)
Report: In vitro cytogenetic investigations of Reg. No. 13 033 in human lymphocytes
BASF AG, Ludwigshafen/Rhein, Germany,
unpublished
BASF RegDoc# 1988/0389

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

Guideline: OECD 473

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), yellow beige powder
Purity: 95.3 %
Batch No.: K 50/16 A
Test substance No.: 86/99
Test system: human lymphocytes

Chloridazon was examined for the ability to cause chromosomal damage in human lymphocytes. The test was performed with and without metabolic activation (S-9 mix from the liver of Aroclor 1254 induced male Sprague-Dawley rats). The test concentrations were chosen on the results of a preliminary cytotoxicity test. The dose selection was based on the quality of the metaphases and not on the mitotic index because the test substance concentrations causing a reduction of the mitotic index at dose levels that severely affect the quality of the chromosome preparation, thus no longer allowing evaluation. The chromosome preparation was performed 24 hours after test substance application. The solvent DMSO (dimethylsulfoxide) was used as negative control while mitomycine (without metabolic activation) and cyclophosphamide (in tests without metabolic activation) were used as positive controls. The test concentrations chosen for the main experiment were 2; 4 and 5 µg/mL without metabolic activation and 1; 2 and 4 µg/mL with S-9 mix. Duplicate cultures were used for all experimental points.

Findings:
No test substance precipitation was reported for the doses selected in this study. According to the results of this study, chloridazon did not lead to an increase in the number of aberrant metaphases including and excluding gaps when compared to the solvent control with or without metabolic activation. The positive controls demonstrated that this system is able to detect mutagens.

Conclusion:
Based on the results of this in vitro cytogenetic study in human lymphocytes chloridazon did not have a chromosome damaging (clastogenic) effect under the test conditions chosen.

B.6.4.1.4 DNA damage and repair

Report: Jagannath D. R., 1989
Mutagenicity test on 88/174, 'Reg. No. 13 033 isomer reduced' in the recombination assays with *Bacillus subtilis* strains H17 (rec+) and M45 (rec-)
Hazleton Laboratories America Inc.; Kensington MD 20895; United States of America
unpublished BASF
RegDoc# 1989/0192

GLP: Yes (laboratory certified by United States Environmental Protection Agency)

Guideline: EPA 84-4, EEC 79/831

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 isomer reduced (chloridazon) - pale yellow powder
Purity: 94.1 %
Batch No.: N 143
Test substance No.: 88/174-1
Test system: recombinant-deficient strain of *Bacillus subtilis*

Isomer reduced chloridazon was examined for its potential to cause cellular DNA damage in the recombinant-deficient strain of *Bacillus subtilis*, M 45 in the presence and absence of metabolic activation (S-9 mix from male Sprague-Dawley rats treated with Aroclor 1254). The H17 (Rec+) and M45 (Rec-) strains are recombination efficient and deficient strains of *Bacillus subtilis*. The damage to DNA is repaired through cellular recombination functions in wild- type (Rec +) cells but not in recombinationless (Rec -) cells. Most agents showing increased lethal activity on Rec- over Rec+ cells may have damaged the DNA. It is highly probable that such DNA damage-provoking agents are mutagenic. Strain M45 carries two independent rec mutations and is sensitive for both frameshift and base-changes. The assay was conducted using three plates per dose level (1 to 10,000 µg/plate) DMSO was used as solvent and solvent control agent while kanamycin was tested as negative control and methylethane sulfonate and diethylnitrosamine were tested as positive controls. The entire test was duplicated (repeat experiment).

Findings:
A test substance precipitation was noted at chloridazon doses of 2,500 µg/plate and higher in all experiments (with and without metabolic activation). In all experiments, no cellular DNA damage as expressed by increased zones of inhibition of the M 45 strain (Rec-) over the H 17 strain (Rec+) was noted for chloridazon, the solvent control and the negative control. The positive control demonstrated that the system was able to detect genetically active compounds.

Conclusion:
Chloridazon did not cause DNA damage to bacterial cells of *Bacillus subtilis* in the presence or absence of metabolic activation under the test conditions chosen.

Report: Shirasu Y. et al., 1976
Mutagenicity testing on Pyramin in microbial systems
The Institute of Environmental Toxicology; Kodaira-shi, Tokyo 187; Japan
unpublished
BASF RegDoc# 1976/012

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

Guideline: Not available for this kind of study

Deviations: Not applicable.

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Test substance was identified as follows:
Chloridazon – former name pyrazon
Purity: 88.3 %
Batch No.: not mentioned
Test system: recombinant-deficient strain of *Bacillus subtilis*

Chloridazon was examined for its potential to cause cellular DNA damage in the recombinant-deficient strain of *Bacillus subtilis*, M 45 in the absence of a metabolic activation system. The H17 (rec+) and M45 (Rec-) strains are recombination efficient and deficient strains of *Bacillus subtilis*. The damage to DNA is repaired through cellular recombination functions in wild- type (Rec +) cells but not in recombinationless (Rec -) cells. Most agents showing increased lethal activity on Rec- over Rec+ cells may have damaged the DNA. It is highly probable that such DNA damage-provoking agents are mutagenic. Strain M45 carries two independent Rec mutations and is sensitive for both frameshift and base-changes. The assay was conducted using one plate per dose level (20 to 2,000 µg/plate). DMSO was used as solvent and solvent control agent while kanamycin was tested as negative control and mitomycine C was tested as positive control.

Findings:
No test substance precipitation was noted in the experiments with chloridazon. No cellular DNA damage as expressed by increased zones of inhibition of the M 45 strain (Rec-) over the H 17 strain (Rec+) was noted for chloridazon, the solvent control and the negative control. This was only noted for the positive control, proving that the system was able to detect genetically active compounds.

Conclusion:
Chloridazon did not cause DNA damage to bacterial cells of *Bacillus subtilis* in the absence of metabolic activation under the test conditions chosen. Tests with metabolic activation were not performed in this study.

Report: Cifone M. A., Myhr B. C., 1986
Evaluation of chloridazon technical (ZNT No. 86/99) in the rat primary hepatocyte unscheduled DNA synthesis assay
Hazleton Biotechnologies Company; Kensington MD 20895; United States of America
unpublished
BASF RegDoc# 1986/257

GLP: Yes (laboratory certified by United States Environmental Protection Agency)

Guideline: Not indicated, however the method used complies to a great extent to OECD 482 (1986)

Deviations: Comparable to OECD 482.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Chloridazon technical, tan powder
Purity: 95.3 %
Batch No.: K 50/16 A
Test substance no.: 86/99
Test system: cultured primary rat hepatocytes

Chloridazon was examined for the ability to cause unscheduled DNA synthesis in cultured primary rat hepatocytes. DMSO was chosen as a solvent. The concentrations initially tested ranged from 0.025 to 1,010 µg/mL. The test concentrations finally evaluated were 5; 10; 25; 50; 101; 252; 504 and 1,010 µg/mL chloridazon. The solvent DMSO was used as negative control while 2-AAF (2-acetyl aminofluorene) was used as positive control. The treatment period was 18 hours. Each treatment, including the positive and negative controls, was performed on five cultures, two of which were used for cytotoxicity measurements; the other three were prepared for microscopic evaluation. UDS was measured by counting nuclear grains and subtracting the average number of grains in three nuclear areas adjacent to each nucleus (background count). The net nuclear grain count was determined for 50 randomly selected cells on each coverslip.

Findings:
The test substance appeared soluble up to 504 µg/mL; higher concentrations resulted in cloudiness. Concentrations > 252 µg/mL were lethal to the cells or caused changes in cell morphology (round cells). None of the 8 treatments ranging from 5 to 1,010 µg/mL caused nuclear labeling different from the solvent control. Furthermore, no dose related trend was evident. In contrast, the positive control caused a large increase in nuclear labeling indicating that the system is able to detect compounds causing DNA damage and repair in primary rat hepatocytes.

Conclusion:
Chloridazon did not induce unscheduled DNA synthesis in primary rat hepatocytes under the test conditions chosen.

B.6.4.2 In vivo testing, somatic cells

B.6.4.2.1 In vivo cytogenetic test

Report: Engelhardt G., Gelbke H.-P., 1987
Report: Cytogenetic study in vivo of Reg. No. 13 033 in mice. Micronucleus test - single oral administration
[redacted]

unpublished
BASF RegDoc# 1987/0424

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

Guideline: OECD 474

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) yellowish-beige powder
Purity: 95.3 %
Batch No.: K 50/16 A
Test substance No.: 86/99
Test animals: NMRI mice

The ability of chloridazon to cause chromosomal damage in vivo was investigated in the mouse micronucleus test. Five male and female NMRI mice/test group were dosed with a single oral (gavage) dose of 150, 300 and 600 mg/kg body weight of chloridazon in 0.5 % aqueous carboxy methyl cellulose (CMC) preparation. A vehicle control and a positive control cyclophosphamide (40 mg/kg bw by gavage) were also tested. Bone marrow for micronuclei examination was prepared 16, 24 and 48 hours after test substance application at the high dose level of 600 mg/kg bw and at 24 h at the low and medium dose level (150 and 300 mg/kg bw). After staining of the preparations 1,000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 1,000 polychromatic erythrocytes were also determined.
The ratio of poly-(PCE's) to normochromatic (NCE's) erythrocytes was determined to assess inhibition of erythropoiesis.

Findings:
Negative and positive control animals did not show any clinical symptoms. Irregular respiration, abdominal position and in some cases tonic-clonic spasms about 30 minutes after treatment were noted in mice treated with 600 mg/kg body weight of chloridazon. The general state of health of these animals appeared to be poor. Mice treated with 150 or 300 mg/kg body weight of chloridazon showed irregular respiration and in some cases abdominal position 30 minutes after dosing. A poor general state of health was noted in a few mice treated with 300 mg/kg body weight. These findings indicate systemic availability of the test compound.
No inhibition of erythropoiesis due to chloridazon administration was evident as can be seen by the ratio of NCE's to PCE's which was not increased. The administration of chloridazon at dose levels from 150, 300 and 600 mg/kg bw did not lead to an increase in the number of polychromatic erythrocytes containing micronuclei. The micronuclei rate was comparable to the negative control, while the positive control had increased rates indicating that the test system is able to detect chromosome damaging (clastogenic) compounds.

Conclusion:
Chloridazon did not induce micronuclei in polychromatic erythrocytes of mice when treated orally even at systemic toxic doses up to 600 mg/kg bw by gavage.

B.6.4.3 In vivo testing, germ cells

B.6.4.3.1 Dominant lethal test

Report: Anonymous, 1975
Study on the mutagenic effect of 5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone on the male mouse following repeated oral administration
[redacted]
unpublished
BASF RegDoc# 1975/008

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

Guideline: Not applicable. Test method described by Röhrborn G. and Vogel F. (Dtsch. Med. Wschr. 92, 2315-2321, 1967).

Deviations: Not comparable to OECD 478.

Acceptability: The study is considered to be supplementary.

And

Report: Merkle, 1981
Statement: Pyramin dominant lethal assay of Feb. 13, 1975
BASF AG, Ludwigshafen/Rhein, Germany,
unpublished
BASF RegDoc# 1981/10088

GLP: No, not subject of GLP regulations

Guideline: Not applicable, not comparable to OECD 478.

Deviations: Not comparable to OECD 478.

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon)
Batch: not indicated
Purity: technical grade active ingredient, at least 84 %
Test substance No.: XXIV/121
Test animals: NMRI mice

Chloridazon was tested for its potential mutagenic effects in the dominant lethal test on male NMRI mice. This test on male mice is an indirect method of determining mutations. The consequences of mutations, which have taken place in the germ cells of male animals, are established in female animals which have been mated with males. The study was performed in accordance with the publication of Röhrborn G. and Vogel F. (Dtsch. Med. Wschr. 92, 2315-2321, 1967).
The study was performed using male mice aged 14 - 16 weeks with an approximate body weight of 33.1 g. The test substance was prepared as an aqueous 0.5 % CMC suspension and dosed orally by gavage on five consecutive days at dose levels of 0 (vehicle control) and 570 mg/kg bw. The doses were selected from an oral LD_{50} determination in this mouse strain. About 20 hours after the 5th test substance administration the male mice were mated with 3 virgin female mouse each for a period of 7 days. Subsequently, the same male mice were mated with three new virgin female mice in 7 other successive mating periods lasting for 7 days each. The females were sacrificed on day 18 after the beginning of each mating week.
All animals were daily examined for clinical signs of toxicity. Body weight of male mice was determined before, once weekly during and at the end of the treatment period. At the end of the 8th mating week all males were sacrificed, dissected and examined macroscopically for any pathological changes of their internal organs.
Females were examined for their conception rate, implantations, number of viable and dead implants, dead fetuses, premature absorptions, and deciduomata (dead implants recognisable with the naked eye on the mucous membrane of the uterus). The mutagenicity index (MI) was calculated as follows:

$$MI = \frac{\text{(total fetuses + deciduomata + premature absorptions)}}{\text{implantations}} \times 100$$

Findings:
The LD_{50} in this strain of mice was determined to be 2,860 mg/kg body weight and mortalities (2/10) were still present at the lowest dose tested (1,000 mg/kg body weight) in the range-finding study.
No clinical symptoms or effects on body weight development were noted in the male NMRI mice treated with 570 mg/kg chloridazon in the Dominant Lethal study. There were no findings at the macroscopic examination of the sacrificed male mice. In female mice conception rate, number of implantations and viable implants were comparable to the control group of this study. There was also no effect on dead implants with the exception of mating week 3.
The increase in number of early resorptions was to a large extend caused by one male only. In the 3 females fertilised by this male all 34 implants died at the early stage of pregnancy (early resorption). The values of the other males were in the normal range. Since in the female animal fertilised by this male the resorption rate did not exceed the normal range either before or after the 3rd mating interval and since the mutagenicity index remained unchanged in the remaining mating intervals, no substance induced effect was attributed to this isolated finding.

Conclusion:
Chloridazon did not have a mutagenic effect in the dominant lethal test in mice when treated orally by gavage for 5 subsequent days at a dose of 570 mg/kg body weight.

B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5)

In Wistar rats in the more recent long-term study body weight gain of 1000 and 2000 ppm dose animals was reduced. At the high dose level (2000 ppm) several red blood cell values were reduced indicating an anaemic effect. In addition, the thromboplastin time was decreased and a few clinical chemical parameters were changed. There were no specific target organs in the long-term rat study. The NOAEL in this study was 300 ppm (13 mg/kg bw/day).

In a high dose supplementary carcinogenicity study in Wistar rats, dietary concentrations of 3000 ppm (females) and 4000 ppm (males) proved to be too high. Body weights in these animals were severely reduced, mortality was increased and there were clinical signs of toxicity such as paresis and ataxia. These dose levels clearly exceeded the maximum tolerated dose. Therefore, the study was prematurely terminated and not used for the purposes of this dossier.

In a carcinogenicity study in Wistar rats the only effect seen at high dose animals (1000 ppm) was reduced body weight. The NOAEL in this study was 300 ppm (13 mg/kg bw/day).

In an older study in Sprague Dawley rats food consumption and body weight gain was impaired in males and females at 4050 ppm. At this dose level red blood cell parameters were reduced mainly in females. In high dose females cholesterol was increased and several organ weights were altered, however, without corresponding histopathological changes.
The increased incidence of muscular atrophy in high dose females correlates with the observation of “prominence of shoulder blades” in these animals. An increased incidence and grade of muscular atrophy was also observed in high dose males. The observations were confirmed in the additional histopathological examination.The only test substance related change in the 1350 ppm group was a slight increase in the incidence and grade of muscular atrophy in females. The fact that similar observations were not found in the more recent long-term studies in rats, in doses up to 2000 ppm may be related to different sensitivities of the different rat strains implied. The NOAEL of this study was 450 ppm (20 mg/kg bw/d).

The overall NOAEL for the chronic rat toxicity was 300 ppm (13 mg/kg bw/d).

Chloridazon was not carcinogenic in any of the long-term studies in rats.

In a more recent carcinogenicity study, B6C3F1 mice treated with a very high dose level (5000 ppm) showed reduced body weight gain and an increase of relative liver weights only. The NOAEL in this study was 1000 ppm equivalent to 134 mg/kg bw in males and 158 mg/kg in females.

In an older long-term study in CFLP mice reduced body weight gain, increased liver weights and histopathological signs of an adaptation of the liver were observed at 20000 ppm. The NOAEL in this study was 4000 ppm, i.e. 351 mg/kg bw in males and 423 mg/kg bw in females

The overall long term NOAEL in mice is 134 mg/kg bw in males and 158 mg/kg in females.

Chloridazon was not carcinogenic in both long-term studies in mice.

The relevant NOAELs are shown in the table below.

Table B.6.5-1: Long-term NOAELs in mg/kg bw

Study	Males	Females
long-term rats	13	18
long-term mouse	134	158

Overall, chloridazon was not oncogenic neither in rats nor in mice. Long-term studies in rats and mice identified kidney, liver and sceletal muscle as target organs.

A summary of the findings of the long-term studies is given in the table below.

Table B.6.5-2: Chloridazon: Summary chronic toxicity and carcinogenicity

Reference / institute	Study / species/ dose levels	Comments	NOAEL
[1993/10819; Mellert W. et al.; 1993c] [redacted]	25 month feeding chronic toxicity Wistar rats 0, 100, 300, 1000 and 2000 ppm	1000 and 2000 ppm: Reduced body weight in both sexes. 2000 ppm: Reduced red blood cell parameters. Decreased thromboplastin time in females. Slightly altered clinical chemical parameters in females. Not oncogenic.	300 ppm 13 mg/kg bw (males) 18 mg/kg bw (females)
[1993/10818; Mellert W. et al.; 1993b] [redacted]	30 month feeding carcinogenicity Wistar rats 0, 100, 300 and 1000 ppm	1000 ppm: Reduced body weight in both sexes. Not oncogenic.	300 ppm 13 mg/kg bw (males) 17 mg/kg bw (females)

Reference / institute	Study / species/ dose levels	Comments	NOAEL
[1977/038; Hunter B. et al.; 1977a] [redacted] AND [1987/0241; Offer M. J., Gopinath C.; 1987] Additional histopathology [redacted]	24 month feeding chronic toxicity and carcinogenicity Sprague-Dawley rats 0, 150, 450, 1350 and 4050 ppm Interim kill after 78 weeks	4050 ppm: Reduced food consumption and body weights in both sexes. Reduced red blood cell parameters in both sexes. Increased cholesterol in females. Altered organ weights (without histopathological changes). Prominence of shoulder blades in females. Increased muscle atrophy. 1350 ppm: Slightly increased muscle atrophy in females. Target organ: skeletal muscle. Not oncogenic.	450 ppm (females) 20 mg/kg bw
[1993/10820; Mellert W. et al.; 1993e] [redacted]	24 month feeding carcinogenicity B6C3F1 mice 0, 200, 1000 and 5000 ppm	5000 ppm: Reduced body weight in both sexes Increased relative liver weight in both sexes. Not oncogenic	1000 ppm 134 mg/kg bw (males) 158 mg/kg bw (females)
[1977/075; Hunter B. et al.; 1977b] [redacted] AND [1987/0243; Cherry C. P., Gopinath C.; 1987] Additional histopathology [redacted]	82 – 97 weeks feeding chronic toxicity and carcinogenicity CFLP mice 0, 160, 500, 4000 and 20000 ppm Interim kill after 26 weeks	20000 ppm: Reduced body weight in both sexes. Increased water consumption in both sexes. Reduced red blood cell parameters in both sexes. Increased alanine aminotransferase in males. Increased liver weights and histopathological signs of adaptation in both sexes. Target organ: liver Not oncogenic.	4000 ppm 351 mg/kg bw (males) 423 mg/kg bw (females)

B.6.5.1 Chronic toxicity rat

Report: Mellert W. et al., 1993(c)
Study of the oral toxicity of Reg. No. 13 033; 95 % in Wistar rats.
Dietary administration for 25 months

[redacted]
unpublished
BASF RegDoc# 1993/10819

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 acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (chloridazon)
Purity: 94.1 %
Batch No.: N 143 (P 5314)
Test substance No.: 88/174-1
Test animals: Wistar rats

Chloridazon was administered to 20 male and 20 female Wistar rats per dose level for 25 months in this chronic toxicity study. The dietary dose levels were 0; 100; 300; 1000 and 2000 ppm.
Feed consumption and body weight was determined once a week for the first 14 weeks. Thereafter, they were determined at 4-week intervals. The animals' state of health was checked each day. Detailed clinical examinations including palpations were performed once a week.
Before the start of the study and at the end of the administration period the animals of the control and high dose group were subjected to ophthalmological examinations.
Clinical-chemical and hematological examinations as well as urinalysis were carried out after about 3, 6, 12, 18 and 24 months of administration.
After 25 months of administration all animals were assessed by gross-pathology and histopathology. Organ weights of selected organs were determined.

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. In a single case the dietary concentration was found to be 85.2 %. A second analysis of this dietary concentration revealed a concentration of 93.6 %. It is concluded that the deviations observed have not negatively influenced the study.

Table B.6.5-3: Test substance intake

Dietary dose level	Test substance intake (mg/kg bw)		
(ppm)	Males	Females	Combined
100	4	6	5
300	13	18	16
1000	43	60	52
2000	88	125	107

There were no test substance related mortalities or signs of clinical toxicity in any of the treatment groups.
Body weight was reduced in males and females of the 1000 and 2000 ppm groups (see table below).

Table B.6.5-4: Body weight after 25 months

Dietary dose level	Body weight (g)	
(ppm)	Males	Females
0	781.0	471.2
100	815.0	437.8
300	794.9	409.0
1000	737.2 (94.4 %)	419.8 (89.1 %)
2000	688.5 (88.2 %)	414.2 (87.9 %)

The body weight of the 2000 ppm males was reduced during most of the study period. In the 1000 ppm males, body weight reduction was observed only during the second half of the study. These differences were not statistically significant but are considered to be test substance related.
The females of the 1000 and 2000 ppm groups showed a body weight reduction compared to the controls, which was statistically significant towards the end of the administration period. Occasionally, differences in body weight were noted between controls and females of the 300 ppm group, statistical significance was obtained in two study weeks towards the end of the study period. The reduction in body weight of females of the two low dose groups is not considered to be test substance related because in the carcinogenicity study [see 1993/10818, Mellert W. et al., 1993b] with this compound, performed in the same laboratory and at the same time, there were no differences between control females and those treated with 100 and 300 ppm. In this study 50 females per dose level were used thus giving a more powerful data base.
There were no test substance related effects on food consumption.

Hematological examinations revealed the following changes at 2000 ppm only:

- Decreased hematocrit.
- Decreased erythrocytes in females.
- Decreased hemoglobin in females.
- Decreased mean corpuscular volume in males.
- Increased anisocytosis.
- Decreased thromboplastin time in females.

Clinical chemical examinations revealed the following changes at 2000 ppm only:

- Increased cholesterol in females.

- Increased calcium in females.
- Increased urea in females.

There were no changes in urinalysis and ophthalmoscopy related to the test substance.
Gross- and histopathological examinations as well as organ weight determinations did not show test substance related effects at any dose levels.
There were no other test substance related lesions in any other test group.
There was no test substance related increase in the incidence of any neoplasia in any group.

Conclusion:
Test substance related effects were limited to reduced body weight in males and females of the 1000 and 2000 ppm groups. A reduction in red blood cell parameters in both sexes was observed at the high dose level only. Slightly altered clinical-chemical parameters were seen in high dose females.

The NOAEL in this study was 300 ppm, equivalent to 13 mg/kg bw in males and 18 mg/kg bw in females.

B.6.5.2 Carcinogenicity rat

Report: Mellert W. et al., 1993(b)
Study of the potential carcinogenicity of Reg. No. 13 033; 95 % in Wistar rats. Dietary administration for 30 months
[redacted]
unpublished
BASF RegDoc# 1993/10818

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

Guideline: OECD 451, EPA 83-2, 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)
Purity: 94.1 %
Batch No.: N 143 (P 5314)
Test substance No.: 88/174-1
Test animals: Wistar rats

Chloridazon was administered to 50 male and 50 female Wistar rats per dose level for 30 months in this carcinogenicity study. The dietary dose levels were 0; 100; 300 and 1,000 ppm. Feed consumption and body weight was determined once a week for the first 14 weeks. Thereafter, they were determined at 4-week intervals. The animals state of health was

checked each day. Detailed clinical examinations including palpations were performed once a week.
At the end of the administration period differential blood counts were determined in all surviving animals. This was also determined in all animals which were killed in extremis during the administration period.
After 30 months of administration all animals were assessed by gross-pathology and histopathology. Organ weights of selected organs were determined.

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. In a single case the dietary concentration was found to be 85.2 %. A second analysis of this dietary concentration revealed a concentration of 93.6 %. It is concluded that the deviations observed have not negatively influenced the study.

Table B.6.5-5: Test substance intake

Dietary dose level	Test substance intake (mg/kg bw)		
(ppm)	Males	Females	Combined
100	4	6	5
300	13	17	15
1000	43	59	51

There were no test substance related mortalities or signs of clinical toxicity in any of the treatment groups.
Body weight was reduced in males of the 1000 ppm group. The reduction, 7 %, was statistically significant from day 35 to 490. In the females of the high dose group no statistically significant differences were observed, however, body weight of this group was numerically lower. In the later phases of the administration period, the differences between control and high dose females were about 6 % (day 490 to day 658).
The effect on body weight in 1000 ppm females is considered to be test substance related because in the chronic toxicity study [see 1993/10819, Mellert W. et al., 1993c], which was performed at the same time and in the same laboratory, also a body weight reduction in females of the 1000 ppm group was observed which was about 11 %.
Moreover, in a high dose supplementary carcinogenicity study in rats, dietary concentrations of 3000 ppm (females) and 4000 ppm (males) proved to be too high. Body weights in these animals were severely reduced, mortality was increased and there were clinical signs of toxicity such as paresis and ataxia. These dose levels clearly exceeded the maximum tolerated dose. Therefore, the study was prematurely terminated and not used for the purposes of this dossier.
There were no test substance related effects on food consumption.
There were no test substance related changes found in the examination of the differential blood counts.
Gross- and histopathological examinations as well as organ weight determinations did not show test substance related effects at any dose levels.
There were no other test substance related lesions in any other test group.
There was no test substance related increase in the incidence of any neoplasia in any group.

Conclusion:
Test substance related effects were limited to reduced body weights in males and females of the 1000 ppm group.

The NOAEL in this study was 300 ppm, equivalent to 13 mg/kg bw in males and 17 mg/kg bw in females.

Chloridazon was not carcinogenic in rats.

Report: Hunter B. et al., 1977(a)
Long-term dietary administration of pyrazon to rats
[redacted]
unpublished
BASF RegDoc# 1977/038

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

Guideline: Not indicated, however, the method used complies to a great extent to OECD 453 (1981)

Deviations: Number of animals too small (40/40 instead of 50/50 per dose group), duration of study too short (103-109 weeks instead of 120 weeks)

Acceptability: The study is considered to be supplementary.

Report: Offer M. J., Gopinath C., 1987
Addendum: Long-term dietary administration of pyrazon to rats. Microscopic pathology addendum report to HRC report BSF 87/7766
[redacted]
unpublished
BASF RegDoc# 1987/0241

GLP: Yes (inspected by the department of health and social security of the government of the United Kingdom)

Guideline: Not indicated, however, the method used complies to a great extent to OECD 453 (1981)

Deviations: Report contains the results of the microscopic examination of additional tissues processed from study BASF RegDoc# 1977/038
Same remarks on study design apply

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Test substance was identified as follows:
Pyrazon Technical (chloridazon), sandy coloured powder

Purity: not mentioned
Batch No.: 942 E679
Test animals: Sprague-Dawley rats

Chloridazon was administered to 55 male and 55 female Sprague-Dawley rats per dose level. This study can be considered to be a combined chronic toxicity and carcinogenicity study (with limited investigations, especially in histopathology) with 40 rats per sex and dose level in the main group (administration for approximately 24 months) and 15 rats per sex and dose level in the satellite groups (interim kill after 78 weeks of administration). The dietary dose levels were 0; 150; 450; 1350 and 4050 ppm.
Feed consumption was determined once a week. Body weight was determined weekly during the first two months and every 14 days thereafter. Water consumption was determined during weeks 5, 13 and 26 in 20 males and 20 females of the high dose and controls groups. The animals' state of health and the occurrence of mortalities were regularly monitored.
Ophthalmoscopic investigations were performed during weeks 6, 12, 26, 52 and 78 in 20 males and 20 females of the high dose and controls groups.
Hematological examinations (packed cell volume, hemoglobin, red blood cell count, MCH, MCHC, MCV, total white blood cell and differential blood cell determination) were carried out using 10 animals per sex of the high dose and control groups during study weeks 0, 5, 12, 25, 52, 77 and 103. Occasionally, hematology was also determined in the 1350 ppm group and compared with additional control animals.
Clinical chemical parameters (urea, glucose, total serum protein, albumine, globulines, alanine aminotransferase, bilirubin, cholesterol, sodium and potassium) were carried out using 5 animals per sex of the high dose and control groups during study weeks 5, 12, 25, 52, 77 and 103. Occasionally, single parameters were also determined in other treatment groups and compared with additional control animals.
Urinalysis was carried out using 5 animals per sex of the high dose and control groups during study weeks 5, 12, 25, 52, 77 and 103.
After 78 weeks all surviving animals from the satellite groups were killed and subjected to detailed macroscopic examinations and organ weight analysis. Tissues were preserved but initially not further processed [for more information see end of section material and methods and 1987/0241 Offer M.J., Gopinath C., 1987].
The main study groups were killed when survival (for each sex determined separately) was approximately 20 %. Thus, after 103 weeks, the remaining 4050 ppm females were killed, after 108 and 109 weeks all remaining animals of the other groups were killed.
After macroscopic pathology, the weight of the following organs was determined:
adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thyroid and uterus.
For the determination of toxic changes to the following organs histopathology was performed on:
Adrenals, bone marrow, brain, caecum, duodenum, eye, heart, ileum, kidneys, liver, lungs, lymph nodes, ovaries, pancreas, pituitary, skeletal muscle, spleen, stomach, testes, thymus, thyroid, urinary bladder and uterus.
The occurrence of tumors was investigated in the following organs:
Adrenals, thyroid, ovaries, liver, spleen, lymph nodes, pituitary gland plus all macroscopically observed lesions suggestive of neoplasia. Abnormalities in the blood smears were confirmed by examination of the bone marrow.

In an addendum [see 1987/0241, Offer M. J., Gopinath C., 1987] to the original report [see 1977/038, Hunter B. et al., 1977a] the results of the following histopathological investigations were reported:

10 male and 10 female control rats and all rats from the 4,050 ppm group main group. The following organs were assessed: aorta, colon, femur, duodenum, jejunum, mammary gland, oesophagus, salivary gland, sciatic nerve, skin, second eye, tong and trachea.
routine samples of kidneys and lungs from all rats (except those which were originally reported).
10 male and 10 female control rats and all rats from the 4050 ppm group satellite animals (killed after 78 weeks of treatment) were reported. These investigations included all standard guideline organs.

Findings:
The correctness of the dietary concentrations was investigated during week 2, 52 and 105. The analytical results confirmed the correctness of the dietary concentrations at all dose levels. From these data it can be derived that the test substance was stable in the food over the entire study period.

Table B.6.5-6: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw)	
	Males	Females
150	5.41	6.7
450	16.79	20.28
1350	50.07	60.33
4050	148.78	193.04

There were no test substance related mortalities, however, high dose females reached the point of 20 % survival five to six weeks earlier than the other groups.
No clinical signs of toxicity were observed during the first 63 weeks of treatment.
At the end of the study a prominence of shoulder blades was observed in females as shown in the table below.

Table B.6.5-7: Clinical observation: prominence of shoulder blades

Dietary dose level	Number of affected animals towards end of study	
(ppm)	Males	Females
0	0	0
150	0	0
450	0	1
1350	0	1
4050	1	14

It was reported that in most of the high dose females observed with “prominence of shoulder blades” depressed forelimb grip reflex was noted.
Body weight gain was statistically significantly reduced in high dose males and females especially during the first 26 weeks of treatment where the differences were 9.3 % in males and 17.6 % in females. Due to the low number of animals surviving until the end of treatment (per study definition: 20 %), body weights at the end of the study have little relevance. Therefore, the data from the 78 week interim sacrifice are presented.

Table B.6.5-8: Body weight satellite animals week 78

Dietary dose level	Body weight in grams (% of control value)	
(ppm)	Males	Females
0	846 (100 %)	565 (100 %)
150	941 (111 %)	616 (109 %)
450	799 (94.4 %)	604 (107 %)
1350	906 (107 %)	556 (98.4 %)
4050	776 (91.7 %)	488 (86.4 %)

Food consumption in high dose males was reduced during the entire treatment period. In females it was reduced from 30 weeks of treatment onwards.
Hematological examinations revealed reduced values at the high dose level only for packed cell volume, red blood cell count and hemoglobin in females during the first year of treatment. In males similar findings were only obtained after 5 weeks of treatment.
The only consistent test substance related change in clinical chemistry was an increase in cholesterol in high dose females only.
There were no changes in urinalysis or ophthalmoscopy in any group.
Taking into account the organ weight determination after 78 weeks and at study termination, the following changes were observed:
A statistically significantly increased absolute and relative liver weight in high dose males at study termination was seen. However, no histopathological changes were seen in the livers. Therefore, the effect on the liver weight in males is of questionable toxicological significance.
In 4050 and 1350 ppm females relative liver weight was statistically significantly increased at study termination. There was no statistically significant increase of absolute liver weight at termination and similarly no statistically significant increase of absolute or relative liver weight in the 78 week interim sacrifice. Moreover, there were no histopathological changes in the liver at any dose level. Therefore, these increases of relative liver weights in females were not assessed to be treatment related and assessed to be not an adverse effect.
In 4050 and 1350 ppm females absolute and relative thyroid weights were statistically significant increased at the 78 week interim sacrifice. At terminal sacrifice only relative thyroid weight was statistically significant increased in the high dose females. Therefore, it is concluded that a test substance related effect on thyroid weight is possible in females treated with 4050 ppm only. There were no histopathological changes in the thyroid at any dose level. Therefore, the effect on thyroid weight is of questionable toxicological significance.
Relative kidney weight in high dose males was statistically significant increased at study termination. There were no histopathological changes noted in the kidneys. Therefore, the effect on kidney weight is of questionable toxicological significance.
In the 4050 and 1350 ppm females relative adrenal weights were decreased at study termination. Absolute adrenal weights were reduced in high dose females only. There were no effects after 78 weeks of treatment. Therefore, it is concluded that a test substance related effect on adrenal weight is possible in females treated with 4050 ppm only. There were no histopathological changes in the adrenals at any dose level. Therefore, the effect on adrenal weight is of questionable toxicological significance.
Muscular atrophy was investigated with special attentions based on the clinical observation "prominence of shoulder blades". The histopathological findings are shown below:

Table B.6.5-9: Scapular muscular atrophy

Finding	0 ppm			150 ppm			450 ppm			1350 ppm			4050 ppm		
	m	f		m	f		m	f		m	f		m	f	
	T*	D**	T	T	D	T	T	D	T	T	D	T	T	D	T
Anterior scapular muscle															
marked															3
moderate													1		4
minimal				1					1		1				3
Incidence	0	0	0	1	0	0	0	0	1	0	1	0	1	0	10
Posterior scapular muscle															
marked														1	4
moderate						1								3	5
minimal	1		1	2	1						2	3		2	1
Incidence	1	0	1	2	1	1	0	0	0	0	1	3	0	6	10
Total number of rats examined	11	11	16	9	12	12	8	14	9	9	10	9	12	10	14

* T = rats killed at study termination
** D = rats that died during the study

The results of these investigations indicate an increased incidence and grade of scapular muscle atrophy in high dose females.

Table B.6.5-10: Thigh muscle atrophy

Finding	0 ppm			150 ppm			450 ppm			1350 ppm			4050 ppm		
	m	f		m	f		m	f		m	f		m	f	
	T*	D**	T	T	D	T	T	D	T	T	D	T	T	D	T
marked											2	1	1	1	1
moderate									1			1	2	3	5
minimal	4	2	2	3	1		5			6		1	6	4	2
incidence	4	2	2	3	1	0	5	0	1	6	2	3	6	8	8
total number of rats examined	12	11	18	10	10	14	10	14	11	9	12	12	11	10	12

* T = rats killed at study termination
** D = rats that died during the study

The results of these investigations indicate an increased incidence and grade of thigh muscle atrophy in high dose males and 1350 and 4050 ppm females. The increased incidence of muscular atrophy correlates with the observation of “prominence of shoulder blades” in high dose females. The slight numerical increase of thigh muscle atrophy in male rats of the 1350 ppm group is unlikely to be treatment related as there was no change in the grade and only minimal atrophy was observed, a common finding also in the controls.
There were no other test substance related histopathological changes in any of the groups.
Chloridazon was not carcinogenic in rats.
In the additional histopathological examinations reported in an addendum [see 1987/0241, Offer M. J., Gopinath C., 1987] to the original report the following findings were obtained:
Minimal to moderate muscular atrophy in the tigh skeletal muscle in males and females of the 4050 ppm groups (both the satellite group killed after 78 weeks and the main group killed at the end of the study. Note, that for the satellite groups only 10 animals of the control group

and 10 animals of the high dose group were investigated. For animals killed at the end of the study only 10 animals of the control group and all animals of the high dose group were investigated, which were not subjected to histopathology in the original study report. Incidences are given in the tables below.

Table B.6.5-11: Effects on the tigh skeletal muscle in animals killed at the end of the study

	Males										Females									
Dose [ppm]	0		150		450		1350		4050		0		150		450		1350		4050	
	D*	T°	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T
Adipose cells in periphal muscle bundles																				
Moderate	-	0	-	-	-	-	-	-	-	3	-	0	-	-	-	-	-	-	-	1
Minimal	-	4	-	-	-	-	-	-	-	5	-	0	-	-	-	-	-	-	-	4
Incidence	-	4	-	-	-	-	-	-	-	8	-	0	-	-	-	-	-	-	-	5
Minimal number of narrowed muscle fibres	-	1	-	-	-	-	-	-	-	6	-	0	-	-	-	-	-	-	-	5
Missing animals/ slices	-	0	-	-	-	-	-	-	-	0	-	0	-	-	-	-	-	-	-	1
Total number of animals	-	10	-	-	-	-	-	-	-	12	-	10	-	-	-	-	-	-	-	10

* D = rats that died during the study
° T = rats killed at study termination
- = not investigated

Table B.6.5-12: Effects on the tigh skeletal muscle in animals killed after 78 weeks (satellite group)

	Males										Females									
Dose [ppm]	0		150		450		1350		4050		0		150		450		1350		4050	
	D*	T°	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T
Adipose cells in peripheral muscle bundles																				
Moderate	-	0	-	-	-	-	-	-	-	0	-	0	-	-	-	-	-	-	-	6
Minimal	-	0	-	-	-	-	-	-	-	6	-	0	-	-	-	-	-	-	-	3
Incidence	-	0	-	-	-	-	-	-	-	6	-	0	-	-	-	-	-	-	-	9
Minimal number of narrowed muscle fibres	-	0	-	-	-	-	-	-	-	8	-	0	-	-	-	-	-	-	-	10
Missing animals/ slices	-	0	-	-	-	-	-	-	-	0	-	0	-	-	-	-	-	-	-	1
Total number of animals	-	10	-	-	-	-	-	-	-	12		10	-	-	-	-	-	-	-	10

* D = rats that died during the study
° T = rats killed at study termination
\- = not investigated

No other non-neoplastic or neoplastic changes were observed in the tissues examined.

Conclusion:
At 4050 ppm food consumption and body weight gain was impaired in males and females. At this dose level red blood cell parameters were reduced mainly in females. In high dose females cholesterol was increased and several organ weights were altered, however, without corresponding histopathological changes.
The increased incidence of muscular atrophy in high dose females correlates with the observation of "prominence of shoulder blades" in these animals. An increased incidence and grade of muscular atrophy was also observed in high dose males. The observations were confirmed in the additional histopathological examinations described in the addendum to the report.
The only test substance related change in the 1350 ppm group was a slight increase in the incidence and grade of muscular atrophy in females. That similar observations were not found in the more recent long-term studies in rats [see 1993/10819, Mellert W. et al., 1993c], also not at a higher dose level (2000 ppm) may be related to different sensitivities of the different implied rat strains. The NOAEL is assessed to be 450 ppm (20 mg/kg bw). Because of deviations from the test protocol the study can be considered only as supportive. The derived NOAEL will not be used for the risk assessment.

Chloridazon is not carcinogenic in rats.

B.6.5.3 Carcinogenicity mouse

Report: Mellert W. et al., 1993(e)
Carcinogenicity study with Reg. No. 13 033; 95 % in B6C3F1 mice.
Administration in the diet for 24 months
[redacted]
unpublished
BASF RegDoc# 1993/10820

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

Guideline: OECD 451, EPA 83-2, 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)
Purity: 94.1 %
Batch No.: N 143 (P 5314)
Test substance No.: 88/174-1
Test animals: B6C3F1 mice

Chloridazon was administered to 50 male and 50 female B6C3F1 mice per dose level for 24 months (main groups) and to 10 males and females for 12 months (satellite groups) in this carcinogenicity study. The dietary dose levels were 0; 200; 1000 and 5000 ppm.
Feed consumption and body weight was determined once a week for the first 14 weeks. Thereafter, they were determined at 4-week intervals. The animals' state of health was checked each day. Detailed clinical examinations were performed once a week.
At the end of the administration period differential blood counts were determined in all surviving animals.
After about 12 months (satellite groups) and 24 months (main groups) of administration all animals were assessed by gross-pathology and histopathology. Organ weights of selected organs were determined.

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

Table B.6.5-13: Test substance intake of main groups

Dietary dose level	Test substance intake (mg/kg bw)		
(ppm)	Males	Females	Combined
200	25	31	28
1000	134	158	146
5000	689	835	762

There were no test substance related mortalities or signs of clinical toxicity in any of the treatment groups.
Body weight was reduced in males and females of the high dose group. At the end of the administration period body weight of 5000 ppm males was 6 % lower than controls (main and satellite groups). In 5000 ppm females, body weight was 13 % reduced in the satellite group and 17 % in the main group.
There were no test substance related changes found in the examination of the differential blood counts.
In the 5000 ppm animals (main and satellite group) relative liver weight was increased.
Gross- and histopathological examinations did not show test substance related effects at any dose level.
There were no other test substance related changes in animals of the 200 and 1000 ppm groups (satellite and main groups).
There was no test substance related increase in the incidence of any neoplasia in any group.

Conclusion:
Test substance related effects were limited to reduced body weights and increased relative liver weight in males and females of the 5000 ppm group.
The NOAEL in this study was 1000 ppm, equivalent to 134 mg/kg bw in males and 158 mg/kg bw in females (146 mg/kg bw for both sexes combined).
Chloridazon was not carcinogenic in mice.

Report: Hunter B. et al., 1977(b)
Long-term feeding of pyrazon to mice
[redacted]
unpublished
BASF RegDoc# 1977/075

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

Guideline: Not indicated, however, the method used complies to a great extent to OECD 453 (1981)

Deviations: Comparable to OECD 453.

Acceptability: The study is considered to be supplementary.

Report: Cherry C. P., Gopinath C., 1987
Long term dietary administration of pyrazon to mice. Microscopic pathology
Huntingdon Research Centre, Huntingdon, United Kingdom
unpublished
BASF RegDoc# 1987/0243

GLP: Yes (inspected by the department of health and social security of the government of the United Kingdom)

Guideline: Not indicated, however, the method used complies to a great extent to OECD 453 (1981)

Deviations: The report contains the results of the microscopic examination of additional tissues processed from study BASF RegDoc# 1977/075

Acceptability: The study is considered to be supplementary.

Material and Methods:

Test material: Test substance was identified as follows:
Pyrazon (chloridazon), brown powder
Purity: not mentioned
Batch No.: 942 E 679 (administered during first 9 weeks) and
Batch No.: 944 E 311 (administered from week 10 until termination)

Test animals: CFLP mice

Chloridazon was administered to 68 male and 68 female CFLP mice per dose level. This study can be considered to be a combined chronic toxicity and carcinogenicity study (with limited investigations, especially in histopathology) with 40 mice per sex and dose level in the main group (administration for 82 – 97 weeks), which were used for the determination of the potential carcinogenicity only. The satellite animals (28 mice per sex and dose level) were partly used for an interim kill after 26 weeks of administration and for all laboratory investigations. The remaining satellite animals were maintained until study termination. These animals were not part of the carcinogenicity screen. The dietary dose levels were 0; 160; 500; 4000 and 20000 ppm. Per cage four mice were housed.

Feed consumption per cage was determined once a week. Body weight was determined weekly. Water consumption was determined during weeks 5, 13 (on ten cages), 26, 65 and 80 on five cages of males and females of all groups. The animals' state of health and the occurrence of mortalities were monitored daily.

Ophthalmoscopic investigations were performed during weeks 6, 12, 26, 52 and 80 in all animals of the high dose and controls groups.

Hematological examinations (packed cell volume, hemoglobin, red blood cell count, MCH, MCHC, MCV, total white blood cell and differential blood cell determination) were carried out using 10 animals per sex of the high dose and control groups during study weeks 0, 6, 12, 26, 52 and 80. Additional samples were obtained from five control males and 10 males from the 4000 ppm group during week 13.

Clinical chemical parameters (plasma urea, plasma glucose, total serum protein, albumine, globulines, alkaline phosphatase, alanine aminotransferase, cholesterol, sodium and potassium) were carried out using 5 animals per sex of the high dose and control groups during study weeks 6, 12, 26, 52 and 80. Additional samples were obtained from five control males and five males from the 4000 ppm group during week 8 for the determination of alkaline phosphatase and alanine aminotransferase. During weeks 52 and 80 additional samples were obtained from five control males and five males from the 4000 ppm group for the determination of alanine aminotransferase.

Urinalysis was carried out using five animals per sex of the high dose and control groups during study weeks 6, 12, 26, 52 and 80.

After 26 weeks eight males and eight females from the satellite groups were killed, with the exception of the 4000 ppm group were six males were killed. The animals were subjected to detailed macroscopic examinations, organ weight analysis and histopathology. The scope of investigations was principally the same as for the terminal kill.

The main study groups were killed if survival was approximately 25 %. Thus after 82 weeks the remaining 160 ppm females were killed, after 87 weeks the 20000 ppm males. When the controls reached the 25 % survival point (females week 93, males week 97) all animals of the remaining groups were killed.
After macroscopic pathology, the weight of the following organs was determined:
Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thyroid and uterus.
For the determination of toxic changes to the following organs histopathology was performed on:
Adrenals, thyroid, ovaries, liver, spleen, lymph nodes and pituitary gland plus all tissues with abnormal macroscopic observations in an attempt to ascertain the cause of death.

The histopathological investigations were restricted to:
1. Control and high dose animals killed after 26 weeks.
2. The liver of the 4000 ppm group of animals killed after 26 weeks.
3. Ten males (9 main group + 1 satellite group) and 10 females (main group) from the controls as well as 17 males (10 main group + 7 satellite group) and 16 females (12 main group and 4 satellite group) from the high dose group killed at termination.
4. The liver of mice from an additional control female, 15 males and 11 females from the 160 ppm group, 12 males and 13 females of the 500 ppm group, 12 males and 8 females from the 4000 ppm group killed at termination (all from the main groups).

The occurrence of tumors was investigated in the following organs:
Adrenals, thyroid, ovaries, liver, spleen, lymph nodes, pituitary gland plus all macroscopically observed lesions suggestive of neoplasia for every animal in the main group. Abnormalities in the blood smears were confirmed by examination of the bone marrow.

In an addendum [see 1987/0243, Cherry C. P., Gopinath C., 1987] to the original report [see 1977/075, Hunter B. et al., 1977b] the results of the following histopathological investigations were reported:
Kidneys and lungs from all mice in all groups.
Trachea, aorta, skeletal muscle, tongue, jejunum, skin, second eye, sciatic nerve and femur from all mice from the control and highest dose group (20000 ppm) killed at termination.

Findings:
The correctness of the dietary concentrations was investigated during weeks 1, 52 and 96. The analytical results confirmed the correctness of the dietary concentrations at all dose levels. Homogeneity was achieved by mixing for 10 minutes. From these data it can be derived that the test substance was stable in the food over the entire study period.

Table B.6.5-14: Test substance intake

Dietary dose level	Test substance intake (mg/kg bw)	
(ppm)	Males	Females
160	14	18
500	44	48
4000	351	423
20000	2215	2490

There were no test substance related mortalities.
No test substance related clinical signs of toxicity were observed.

Body weight gain was reduced in high dose males during the first 52 weeks of the study and in females during the first 26 weeks of treatment.

Table B.6.5-15: Body weight (g) of control and high dose animals at weeks 26, 52 and at termination

Study week	Males		Females	
Dose level	0 ppm	20000 ppm	0 ppm	20000 ppm
Week 26	53	49	41	39
Week 52	59	52	49	45
Termination	57.2	47.3	52.9	45.7

Food consumption was not affected. Water consumption was increased in high dose males and females.
Hematological examinations revealed reduced values for packed cell volume, red blood cell count, hemoglobin and MCHC in high dose males at week 12 only. Thereafter, no test substance related effects were noted in any of the animals.
The only consistent test substance related change in clinical chemistry was an increase in alanine aminotransferase in high dose males only.
There were no changes in urinalysis or ophthalmoscopy in any group.
In the animals killed after 26 weeks of administration the following changes were seen:
A statistically significant increase in absolute and relative liver weights in high dose males and relative liver weight in high dose females was observed. These changes were associated with minimal enlargement of centrilobular hepatocytes with granular eosinophilic cytoplasm and the absence of centrilobular fat.
A small but statistically significant increase in absolute and relative spleen and ovary weight in high dose females was observed. These changes were not associated with pathological findings.
In the animals killed at termination the following changes were observed:
A statistically significant increase in relative liver weights in high dose males and females was observed. These changes were associated with granular eosinophillic cytoplasm, without hepatocellular enlargement.
In decedents from the main groups eosinophilic cytoplasm was found in 8 out of 29 males and 12 out of 26 females of the 20000 ppm group and in 7 out of 20 males and 3 out of 27 females of the 4000 ppm group. It was not found in any other group. This change, however, was not observed in terminal kill 4000 ppm animals. Liver weights in these animals were also similar to controls. Therefore, these changes are not definitively associated with the administration of chloridazon and lacking any other liver changes at the most a sign of adaptation but not an adverse effect.
There were no other test substance related histopathological changes in any of the groups.
Chloridazon was not carcinogenic in mice.

In the additional histopathological examinations reported in an addendum [see 1987/0243, Cherry C. P., Gopinath C., 1987] no test substance related neoplastic or non neoplastic changes were obtained.

Conclusion:
At 20000 ppm body weight gain was impaired in males and females. At this dose level red blood cell parameters were reduced in high dose males at week 12 only. Alanine aminotransferase was increased in high dose males. Liver weights were increased in high dose animals at

week 26 and termination. Microscopically the increased liver weight at 20000 ppm was associated with signs of adaptation.
The only possible test substance related change in the 4000 ppm group was a slight increase in eosinophilic cytoplasm in a few animals which died before study termination.
The NOAEL is assessed to be 500 ppm in males (44 mg/kg bw) and females (48 mg/kg bw). As the study is considered supportive the NOAEL will not be used in the risk assessment.

Chloridazon is not carcinogenic in mice.
Studies on delayed neurotoxicity potential of chloridazon were considered to be not relevant and were not conducted. It is noted that the structure of chloridazon is not similar or related to that of compounds known to be capable of inducing delayed neurotoxicity.

With regard to neurotoxicity no indications of an acute or subchronic neurotoxic effect could be derived from the toxicity studies performed with chloridazon. Chloridazon showed no clinical signs in any of the toxicity studies which could be attributed to neurotoxicity, and no indications on histopathological changes on the central or peripheral nerve system were seen in the short-term, long-term or reproductive/developmental studies in rats, mice and dogs.

B.6.6 Reproductive toxicity (Annex IIA 5.6)

Data on reproduction toxicity and developmental toxicity studies with chloridazon are summarised in Table B.6.6-1.

In a recent study chloridazon was administered via the feed to Wistar rats for two consecutive generations at dose levels of 0, 100, 400 and 1600 ppm. Effects on parental animals (body weight, body weight gain, increased triglycerides and liver weights, hepatocyte swelling and lipid deposits) were noted at the high dose. The only finding in pups was a reduced body weight and body weight development at this dose level. No effects were noted at the low and mid dose level for parental animals and their offspring. The NOAEL for systemic toxicity in parental animals and offspring was 400 ppm (37 mg/kg bw). There was no indication of an impairment of fertility in both sexes at all dose levels, thus the NOAEL for reproductive performance and fertility was 1600 ppm (about 148 mg/kg bw/day).

In an older multigeneration study, where CFY rats had been treated via the feed over three consecutive generations with doses of 0, 150, 440 and 1350 ppm, no effects on parental animals and pups were noted at any dose level. Similarly there were also no adverse effects on reproduction and fertility parameters. The dose levels were in the same range as in the above-mentioned 2-generation study, however, the scope of examination was not as extensive. The relevant NOAELs therefore should be derived from the more recent 2-generation study [see 1993/10632, Hellwig J., Hildebrand B., 1993].

In a pre-, peri-, postnatal feeding study in NMRI mice according to FDA method (segment II and III) no effects were noted in the prenatal phase (segment II) in dams treated with 0, 5000 and 10000 ppm. There was also no indication of embryo-/fetotoxicity and no malformations were noted. In the peri-/postnatal phase (segment III) with identical doses the pup vitality index was slightly reduced at 10000 ppm (1742 mg/kg bw) in the test group where dams received chloridazon throughout pregnancy up to day 21 post partum. No such effect was noted in the test group when the dams had only been treated during the gestation period. In addition there was a slight increase in liver weights of dams and pups. No other adverse effects were

noted for dams and pups including pup examination for anomalies, variations or malformations. No adverse effects were noted at the 5000 ppm dose level (905 mg/kg bw).

When chloridazon was administered by gavage to Wistar rats in a prenatal toxicity study maternal toxicity was marked at the high dose of 250 mg/kg bw leading to reduced food consumption, impairment of body weight and body weight development (including corrected body weight gain), and clinical symptoms (piloerection). At 50 mg/kg bw there was a slight effect on food consumption and body weight gain. There was no indication that the administration of chloridazon causes embryo-/fetotoxicity or malformations at any dose levels.

When chloridazon was administered by gavage to Chinchilla Russian rabbits, maternal toxicity at the doses of 165 mg/kg bw and 495 mg/kg bw in form of impairment of food consumption and body weight was observed. The NOAEL was 55 mg/kg bw. No adverse effects on embryo-/fetal development - including the occurrence of malformations - could be noted even at the high dose of 495 mg/kg bw which showed clear maternal toxicity.

The following NOAELs have been established for reproduction toxicity including fertility and prenatal toxicity of chloridazon:

Reproduction toxicity studies

Systemic toxicity/parental animals:	37 mg/kg bw (400 ppm, rat)
Systemic toxicity/developmental toxicity pups:	37 mg/kg bw (400 ppm, rat)
Fertility:	148 mg/kg bw (1600 ppm rat)

Prenatal toxicity studies

NOAEL maternal toxicity:	10 mg/kg bw (rat)
NOAEL embryo-/fetotoxicity:	250 mg/kg bw (rat)
NOAEL malformations:	250 mg/kg bw (rat)

Table B.6.6-1: Chloridazon - summary table reproduction toxicity and prenatal toxicity

Institute/year/ reference	Study type/species/ dose levels (vehicle)	Comments	NOAEL
[1993/10632; Hellwig J., Hildebrand B.; 1993] [redacted]	2 generation study Wistar rats 0, 100, 400 and 1600 ppm in the diet	1600 ppm: Effects on body weight and body weight gain, triglycerides, liver (weight and histology) in dams. Reduced pup body weight and growth. No adverse effects below and no effect on reproductive function.	Parental animals: 400 ppm (37 mg/kg bw) Systemic toxicity offspring: 400 ppm (37 mg/kg bw) Reproductive function: 1600 ppm (148 mg/kg bw)
[1977/076; Palmer A. K., Allen T. R.; 1977] [redacted]	3 generation study in CFY rats 0, 150, 450 and 1350 ppm in the diet	Study not acceptable No toxic effects, no effects on reproduction, limited number of examinations; NOAEL should be derived from the 2-generation study	Parental animals: 1350 ppm (approx. 120 mg/kg bw). Systemic toxicity offspring: 1350 ppm (approx. 120 mg/kg bw). Reproductive function: 1350 ppm (approx. 120 mg/kg bw)

Institute/year/ reference	Study type/species/ dose levels (vehicle)	Comments	NOAEL
[1975/009; Peh J., Hofmann H. T.; 1975] [redacted]	Pre-, peri-, postnatal toxicity in NMRI mice 0, 5000 and 10000 ppm in the feed	Study supplemental 10000 ppm: Increased liver weights in dams and pubs when dams were treated until day 18 p.p. Reduced viability index of pups when dams were treated until day 18 p.p.	Parental animals: 5000 ppm (905 mg/kg bw) Systemic toxicity offspring: 5000 ppm (905 mg/kg bw) No embryo-/fetotoxic effects and malformations
[1990/0163; Hellwig J., Hildebrand B.; 1990] [redacted]	Prenatal toxicity in Wistar rats: 0, 10, 50 and 250 mg/kg bw in aqueous CMC preparation by gavage	250 mg/kg bw: Effects on food consumption, body weight, body weight gain and clinical symptom's (piloerection) in dams. 50 mg/kg bw: Initial slight effect on food consumption and very slight effect on body weight gain in dams.	NOAEL maternal toxicity: 10 mg/kg bw NOAEL prenatal toxicity: 250 mg/kg bw NOAEL anomalies: 250 mg/kg bw
[1987/0412; Becker H. et al.; 1987] [redacted]	Prenatal toxicity study in Chinchilla Russian rabbits (range-finding study) 0, 100, 250 and 500 mg/kg bw in 4 % aqueous Na-CMC by gavage	Study indicates that the dose range selected would be suitable for the main study	Range-finding study – NOAEL should be derived from main study [see 1987/0413 Becker H. 1987]
[1987/0413; Becker H.; 1987] [redacted]	Prenatal toxicity study in Chinchilla Russian rabbits 0, 55, 165 and 495 mg/kg bw in 4 % aqueous Na-CMC by gavage	495 mg/kg bw: Effects on food consumption, body weight, body weight gain in dams 165 mg/kg bw: Effects on food consumption, body weight and body weight gain in dams	NOAEL maternal toxicity: 55 mg/kg bw NOAEL prenatal toxicity: 495 mg/kg bw NOAEL anomalies: 495 mg/kg bw

CMC = carboxymethylcellulose

B.6.6.1 Multigeneration studies in mammals

Report: Hellwig J., Hildebrand B., 1993
Reproduction toxicity study with Reg. No. 13 033; 95 % in rats. - Continuous dietary administration over 2 generations (2 litters in the first and 1 litter in the second generation)
[redacted]
unpublished
BASF RegDoc# 1993/10632

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

Guideline: OECD 416, EPA 83-4, 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); 95 %, brown powder
Batch: N 143
Purity: ≥ 94.1 %
Test substance No.: 88/174-1
Test animals: Wistar rats (F0 parental generation)

Chloridazon was administered to groups of 24 male and 24 female Wistar rats (F0 parental generation) in the feed at concentrations of 0; 100; 400 and 1600 ppm [see Table B.6.6-2].
The doses were selected upon the results from a previously performed reproduction study in CFY rats [see point 6.6.1 below, #1977/076, Palmer A. K., Allen T. R., 1977] in addition to short-term/chronic studies of chloridazon in Wistar rats. The age of the F0 Generation animals at the beginning of treatment was 35 ± 1 day. One female was mated with one male overnight for a period of maximum 3 weeks at least 70 days after the beginning of treatment to produce the F1 litter [see Table B.6.6-3].
The F0 generation was remated to produce a second litter. From the F1a pups 24 males and 24 females/dose group were selected as F1 parent generation to produce the F2 generation. They received the test substance at least 98 days before mating.
The examination of parental animals included monitoring for clinical symptom's/mortalities, food consumption, body weight development, clinical chemistry, mating and reproductive performances. Pathological examination was performed by gross inspection as well as extensive histopathological examination with special attention to the organs of the reproductive system. Pups were sexed and monitored macroscopically at necropsy (external and organ findings), stillborn pups and pups that died intercurrently were additionally examined for any skeletal findings.

Table B.6.6-2: Study design - 2-generation feeding study in Wistar rats

Dose levels	0 (Control)	100 ppm	400 ppm	1600 ppm
F0 generation	Treatment at least 70 days prior to mating to produce F1a pups			
No. of males	24	24	24	24
No. of females	24	24	24	24
F1a generation - parent animals:	Treatment at least 98 days prior to mating to produce F2 pups (only 1 litter) until sacrifice. Infertile animals were remated with a fertile partner.			
No of males	24	24	24	24
No of female's	24	24	24	24
F1a/b and F2a generation pups:	Reared until day 4 post partum where 4 males and females were selected for further rearing until day 21 post partum. The rest was sacrificed and examined macroscopically (external and organs). Stillborn pups or pups that died as well as pups showing remarkable findings during rearing or abnormalities during macroscopic inspection were examined according to the method of Wilson (head) and Dawson (modified) for their skeleton.			

Findings:
It was shown that the test substance was stable over the entire period of the study and its homogeneity was proven. The stability of chloridazon in the food over a period of 32 days was proven prior to dosing. Correctness of dietary concentrations was proven by analytical determination at several intervals throughout the study. Test substance intake is given in the table below.

Table B.6.6-3: Mean daily test substance intake - 2-generation feeding study in Wistar rats (mg/kg bw)

Dose level (ppm)	0 (control)	100 ppm	400 ppm	1600 ppm
F0 males	0	9.2	37.1	146.5
F0 females (premating)	0	9.9	39.3	155.5
F0 females (F1a litter) gestation period	0	8.5	33.3	132.5
F0 females (F1a litter) lactation period	0	15.0	57.4	233.9
F0 females (F1b litter) gestation period	0	8.1	31.7	123.9
F0 females (F1b litter) lactation period	0	14.0	53.4	219.5
Average (F0 males/females) premating period	0	9.6	38.2	151.0
F1 males	0	8.5	34.8	136.6
F1 females - premating period	0	9.7	38.4	153.9
F1 female's - gestation period	0	8.0	31.5	131.2
F1 females (F2-litters) - lactation period (day 0-14 post partum only)	0	13.9	56.0	231.8
Average (F1 males/females) premating period	0	9.1	36.6	145.3
Average (F0 and F1 males/females) premating period	0	9.3	37.4	148.1

At the high dose level (1600 ppm) effects on bodyweight and bodyweight gain, clinical chemistry, organ weights and histopathological changes can be assessed as slight and are listed in detail [see Table B.6.6-4]. The increased absolute liver weight in F0 females at 400 ppm, in the absence of an increase of relative weight and any other finding (e.g. histopathology or clinical chemistry) which might indicate an effect on this organ at 400 ppm, is not regarded to represent an adverse effect. The only effect noted in the offspring was a lower body weight and retarded growth (bodyweight gain) in F1a and F1b pups at 1600 ppm, indicating a

systemic toxic effect, which was also observed in parental animals at this dose level. There were no effects on fertility parameters at any dose level.

Table B.6.6-4: Parental/offspring findings – 2 generation feeding study in Wistar rats

Dose level	0 (control)	100 ppm	400 ppm	1600 ppm
Parental data				
Clinical data				
Body weight (F0 males, week 29 in g):	576.6	569.5	565.0	550.3***
Body weight gain (F0 males week 0-29 in g):	436.3	429.7	425.5	411.4***
Clinical chemistry				
Triglycerides (mmol/L)				
F0 males (peripheral blood)	7.5	6.6	6.8	5.1
F1 males (peripheral blood)	8.2	6.6	8.2	5.0
F0 females (serum)	2.7	2.5	2.8	1.7*
Organ weights				
Absolute liver weight (g) F0 female's:	10.3	10.5	11.1*	12.0**
Relative liver weight				
F0 females	3.2	3.2	3.4	3.7*
F1 females	3.6	3.6	3.6	4.0**
Histopathology				
Hepatocytes (hydropic swelling):				
F0 males	0/24	0/24	0/23	9/24
F0 females	0/23	0/24	0/24	4/24
F1 males	0/24	0/24	1/24	10/24
Liver: lipid deposits:				
F0 males	23/24	23/24	20/23	17/24
F0 females	13/23	16/24	18/24	6/24
F1 males	19/24	16/24	22/24	13/24
F1 female's	6/24	19/24	8/24	3/24
Offspring data				
Pup bodyweight (males and females day 21 in g):				
F1a	52.5	52.9	53.0	50.0
F1b	54.3	55.5	53.0	50.7*
Pup body weight change (males and females days 4-21 in g):				
F1a	43.1	43.5	43.1	40.5*
F1b	45.0	45.7	43.6	41.6*

* $p \leq 0.05$ / ** $p \leq 0.01$ / *** $p \leq 0.05$ or ** $p \leq 0.01$ at some examination intervals

Conclusion:
The following NOAELs have been determined for this 2-generation study in Wistar rats: NOAEL for general toxicity/systemic effects for male and female parental animals and offspring: 400 ppm (about 37 mg/kg bw/day), NOAEL for reproductive performance and fertility: 1600 ppm (about 148 mg/kg bw/day).

Report: Palmer A. K., Allen T. R., 1977
Effect of pyrazon on reproductive function of multiple generations in the rat

[redacted]
unpublished
BASF RegDoc# 1977/076

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

Guideline: Not indicated, however, the method used complies to a great extent to OECD 416

Deviations: Purity of test substance not stated. No clinical signs reported. No histopathlogy.

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (pyrazon; chloridazon)
Batch: Nos. 942 E679 and 944 E311
Purity: not mentioned
Test animals: young CFY rats (F0 parental generation)

Chloridazon was administered to groups of 20 male and 20 female young CFY rats (F0 parental generation) in the feed at concentrations of 0; 150; 450 and 1350 ppm [see Table B.6.6-5]. The animals were mated 1:1 (males/females) for a period of maximum 19 days at least 60 days after the beginning of treatment to produce the F1 litter [see Table B.6.6-6]. From the F1 respectively F2 pups 20 males and 20 females/dose group were selected as F1 or F2 parent generation to produce the F2 or F3 generation. They received the test substance at least 60 days before mating.

The following parental parameters were measured for the parental animals:
- food consumption
- body weight/body weight change
- mortality and clinical symptoms
- test substance intake
- pregnancy rate (fertility)
- mating performance
- gestation period
- macroscopic pathology

The following pup/litter parameters were measured:
- litter size
- litter weight
- pup weight
- pup mortality
- macroscopic examination

Table B.6.6-5: Study design - 2-generation feeding study in CFY rats

Dose levels	**0 (Control)**	**150 ppm**	**450 ppm**	**1350 ppm**
F0 generation – parent animals:	Treatment of young rats for at least 60 days prior to mating to produce F1 pups (1 litter) until sacrifice (day 21 post partum).			
No of males	20	20	20	20
No of female's	20	20	20	20
F1 generation – parent animals:	Treatment at least 60 days prior to mating to produce F2 pups (1 litter) until sacrifice (day 21 post partum).			
No of males	20	20	20	20
No of female's	20	20	20	20
F2 generation – parent animals:	Treatment at least 60 days prior to mating to produce F3 pups (1 litter) until sacrifice (day 21 post partum).			
No of males	20	20	20	20
No of female's	20	20	20	20
F1, F2 and F3 generation pups:	Reared until day 21 post partum.			

Findings:
The correctness of the dietary concentrations was confirmed by analysis. Chloridazon was demonstrated to be stable in the food in several studies.
Test substance intake was not given in the report, therefore for the purpose of this dossier it was calculated. The calculations were based on the analytical dietary test substance concentrations, the reported food consumption (during the premating period) and body weight; the calculations were performed for the premating period of the F0, F1 and F2 generations, [see Table B.6.6-6].

Table B.6.6-6: Test substance intake (mg/kg/day) premating period – 3 generation study in CFY rats

	Males				**Females**			
Test group (ppm)	0	150	450	1350	0	150	450	1350
Test substance intake (g)								
F0 generation	-	11	46	114	-	12	50	130
F1 generation	-	14	34	106	-	14	37	118
F2 generation	-	12	39	115	-	14	45	131
Average F0/F1/F2		12	39	112		13	44	126

There were no mortalities or clinical symptoms at any dose levels. No consistent trend was noted for food consumption and bodyweight development over 3 generations treated with chloridazon at different dose levels. Thus no test substance related effect was assumed.
There were also no effects on mating performance, pregnancy rate and gestation period. Terminal autopsy showed no macroscopic changes attributable to treatment. Litter parameters such as total litter loss, litter size, pup mortality, litter and mean pup weight were also not affected by the test substance. The few abnormalities noted in the study were within the historical range and not related to treatment.

Conclusion:
Based on the results of this study, the NOAEL for parental parameters and the offspring is 1350 ppm (111 mg/kg bw males, 126 mg/kg bw females) in CFY rats. As the study does not comply with current requirements the NOAEL should not be used in the risk assessment.

Report: Peh J., Hofmann H. T., 1975
Trial report on the pre-, peri- and postnatal toxicity of 5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone in the mouse
[redacted]
unpublished
BASF RegDoc# 1975/009

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

Guideline: In accordance with FDA Guidelines (1966), section II and III

Deviations: Guideline not available.

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Test substance was identified as follows:
Reg. No. 13 033 (pyrazon; chloridazon)
Batch: not indicated
Purity: technical active ingredient
Test substance No.: XXIV/121
Test animals: NMRI mice

Chloridazon was examined for pre-, peri- and postnatal toxicity in NMRI mice. The dams (10 or 21/test group) received 0; 5000 or 10000 ppm chloridazon via the feed from day 0 post coitum (p.c.) until day 18 post coitum or day 21 post partum. For details of treatment of the different test group [see Table B.6.6-7].

Table B.6.6-7: Dosing scheme – pre-, peri-, postnatal gavage toxicity study in NMRI mice

Group	No. of mice	Dose (ppm)	Dose (ppm)
0	21	0	Untreated control for group 1
1	21	5000	Day 0 p.c.- day 18 p.c.
2	10	0	Untreated control for group 3, 4
3	10	5000	Day 0 p.c.- day 18 p.c. then no treatment until day 21 p.p.
4	10	5000	Day 0 p.c.- day 21 p.p.
5	21	0	Untreated control for group 6
6	21	10000	Day 0 p.c.- day 18 p.c.
7	10	0	Untreated control for group 8, 9
8	10	10000	Day 0 p.c.- day 18 p.c. then no treatment until day 21 p.p.
9	10	10000	Day 0 p.c.- day 21 p.p.

p.c. – post coitum / p.p. – post partum

FDA Section II examination (prenatal investigation) of dams included measurement of food intake and body weight at certain intervals as well as monitoring for mortalities and clinical symptoms. Post mortem studies (day 18 p.c.) included examination for implantation's, resorptions, living/dead fetuses, external examination of fetuses as well as determination of placental weights and fetal weights and length.

FDA Section III examination (peri-, postnatal investigation) of dams (test groups with continuous treatment after birth or solely in utero treatment) included measurement of food intake and body weight at certain intervals as well as monitoring for mortalities and clinical symptom's. Delivery data such as size of litter, viable/dead pups, and vitality index were recorded. Post mortem examination of the mother animals included macroscopic pathological examinations, determination of implantations, resorptions, conception rate, selected organ weights (absolute and relative). Pups were examined for mortality, weight gain evaluation of bone structure and organs in pups that died. A swimming test with the pups was performed on day 21 p.p. Post mortem examination of pups (day 21.p.p.) included macroscopic pathological examinations, determination of selected organ weights (absolute and relative) and examination of the head (Wilson's method) and bone structure (Dawson's method).

Findings:
Chloridazon was demonstrated to be stable in the food in several studies.
Test substance intake was not given in the report, therefore for the purpose of this dossier it was calculated for days 0 – 18 p.c., based on the reported food consumption and body weight [see Table B.6.6-8].

Table B.6.6-8: Test substance intake (mg/kg/day) of dams day 0-18 p.c. Pre-/peri-/postnatal feeding study in NMRI mice

Dose level in the feed	**5000 ppm**	**10000 ppm**
Intake mg/kg/day	905	1742

FDA Section II (prenatal investigation)
No clinical symptoms or mortalities were noted in dams treated with chloridazon. No effect was noted on food consumption and body weight development. No adverse effects were noted at cesarean section in the above-mentioned parameters of the dams and fetuses that could be attributed to the administration of the test substance.

FDA Section III (peri-, postnatal investigation)
No clinical symptoms or mortalities were noted in dams treated with chloridazon. No effect was noted on food consumption and body weight development with the exception of animals receiving compound free feed from day 18 p.c. onwards. These animals consumed less feed than those who were treated continuously until study termination (day 21 p.p.). This finding is not considered to be relevant.
Litter parameters were not affected by chloridazon treatment. There were no remarkable findings in pup examinations with the exception of a lower survival rate in pups whose dams received 10000 ppm throughout the study (until day 21 p.p). Postmortem examination of all dams and pups revealed no macroscopic pathological changes. Increased liver weights in pups were noted at the 10000 ppm dose level. Dams of this group (treatment until day 21 p.c.) were found to have increased relative liver weights.
There was no indication that chloridazon caused malformations, retardation's or variations in the offspring.

Table B.6.6-9: Maternal/pup findings – pre-, peri-, postnatal gavage toxicity study in NMRI mice

	0 ppm	5000 ppm	10000 ppm
Absolute liver weights (g):			
Treatment until 18 days p.c.:			
Dams	1.54 / 1.53*	1.60	1.38
Male pups	0.273 / 0.267	0.254	0.318
Female pups	0.276 / 0.308	0.242	0.333
Treatment until 21 days p.p.:			
Dams	1.54 / 1.53	1.59	1.89
Male pups	0.273 / 0.267	0.321	0.336
Female pups	0.276 / 0.308	0.286	0.335
Relative liver weights:			
Treatment until 18 days p.c.:			
Dams	5.99 / 5.71	6.13	5.50
Male pups	4.113 / 4.015	4.098	4.265
Female pups	4.278 / 4.186	4.015	4.488
Treatment until 21 days p.p.:			
Dams	5.99 / 5.71	6.55	7.20
Male pups	4.113 / 4.015	4.506	4.716
Female pups	4.278 / 4.186	4.154	4.551
Pup survival mice (vitality index). Continuous treatment until day 21 p.p. (the value of the resp. control group is given in brackets)		84.9 % (90.0 % control group 2)	73.2 % (95.4 % control group 7)

* a/b = control value 5000 ppm/control value 10000 ppm

Conclusion:
Chloridazon was shown to have no teratogenic effects.

The following NOAELs have been identified in this study:

FDA Section II (prenatal treatment):
NOAEL dams: 10000 ppm (1742 mg/kg bw)
NOAEL embryo-/fetotoxicity: 10000 ppm

FDA Section III (peri-/postnatal treatment)
NOAEL dams: 5000 ppm (905 mg/kg bw)
NOAEL pup toxicity: 5000 ppm
NOAEL malformations, retardation's, variations: 10000 ppm (1742 mg/kg bw)

B.6.6.2 Developmental toxicity

B.6.6.2.1 Rat

Report: Hellwig J., Hildebrand B., 1990
Report: Study of the prenatal toxicity of Reg. No. 13 033 in rats after oral administration (gavage)
[redacted]
unpublished
BASF RegDoc# 1990/0163

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

Guideline: OECD 414, EPA 83-3, 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), brown powder
Batch: N 143
Purity: 94.1 %
Test substance No.: 88/174-1
Test animals: Wistar rats

Chloridazon was examined for its prenatal toxicity in Wistar rats. The dams (25/test group) were treated from day 6 through day 15 post coitum (p.c.) with chloridazon doses of 0 (control treated with the vehicle double distilled water with 0.5 % carboxymethylcellulose), 10, 50 and 250 mg/kg body weight by gavage and a constant dosing volume of 5 mL/kg body weight [see Table B.6.6-10].
The doses have been selected using the results of a preliminary study in the same strain of rats. The animals were observed for food consumption and body weight gain regularly throughout the study period. Their state of health was checked daily. On day 20 p.c., all females were sacrificed and assessed by gross pathology. The fetuses were dissected from the uterus, sexed weighed and further investigated for any external, soft tissue and/or skeletal findings.

Table B.6.6-10: Dosing scheme – prenatal toxicity gavage study in Wistar rats

Group number	Number of females mated/pregnant	Concentration (mg/100 mL)	Dose volume	Dosage level
0	25/23	-	5 mL/kg*	0 mg/kg bw*
1	25/24	200	5 mL/kg	10 mg/kg bw
2	25/24	1000	5 mL/kg	50 mg/kg bw
3	25/23	5000	5 mL/kg	250 mg/kg bw

*treated with double distilled water containing 0.5 % carboxymethylcellulose (CMC)

Findings:
Homogeneity and stability of the test substance was proven throughout the study period. The test substance formulation was established over a period of 42 days at room temperature. The correctness of the prepared concentrations and their homogeneity were also proven.
Maternal data are compiled in Table B.6.6-11 and cesarean section/fetal results in Table B.6.6-12. There was a clear effect on food consumption of the dams during the treatment period (days 6-15 p.c.) and a marked influence on body weight and bodyweight gain during most parts of the treatment. At the beginning of treatment (days 6-8 p.c.) even a body weight loss was noted. The corrected body weight gain was also reduced. Piloerection was noted as a clinical symptom in most of the dams. At the mid dose (50 mg/kg bw) there was only an initial slight effect in food consumption (days 6-8 p.c.) and a marginal impairment of body

weight gain (days 6-8) in the dams. No maternal toxicity was noted at the low dose of 10 mg/kg bw.

Table B.6.6-11: Maternal data - prenatal gavage toxicity study in Wistar rats

Dose (mg/kg bw)	0	10	50	250
Mated females on study	25	25	25	25
Pregnant females on study	23	24	24	23
Mortality of dams	0	0	0	0
Clinical symptoms:	-	-	-	Piloerection
Food consumption: treatment day 6-15 p.c. in g/animal/day and %	25.0 100 %	25.2 101 %	24.0 96 %	20.4 82 %
Body weight (day 8 p.c. in % of control)	100	99.9	99.8	97.2**
Body weight gain (days 6-15 in g and % of control)	50.2 100 %	51.8 103 %	47.9 95 %	40.8** 81 %
Corrected body weight gain (net weight change from day 6 in g and %)	42.0 100 %	48.0 114 %	43.1 103 %	34.3 * 82 %

* p<0.05 **p<0.01

There was no effect on embryo-/and fetal development at any of the investigations made in this study. This includes also malformations.

Table B.6.6-12: Data at cesarean section/fetal examination prenatal toxicity gavage study in Wistar rats

Dose (mg/kg bw)	0	10	50	250
Females mated	25	25	25	25
Pregnant dams	23	24	24	23
Pregnant at cesarean section (%)	92	96	96	92
Aborted	0	0	0	0
Premature birth	0	0	0	0
Dams with viable fetuses	23	24	24	23
Dams with all resorptions	0	0	0	0
Female mortality	0	0	0	0
Corpora lutea (mean)	15.4	15.6	15.6	15.3
Implantation sites/(mean)	14.4	14.2	14.6	14.4
Preimplantation loss (mean)	6.4	8.9	6.7	5.7
Postimplantation loss (mean)	5.4	7.5	9.4	7.4
Total resorptions (mean)	0.8	1.1	1.3	1.0
Early resorptions (mean)	0.8	1.1	1.1	1.0
Late resorptions (mean)	0	0	0.2	0.1
Dead fetuses	0	0	0	0
Live fetuses (mean)	13.6	13.1	13.3	13.4
Placental weights in g (mean)	0.41	0.42	0.41	0.42
Fetal weights in g (mean)	3.9	4.0	4.0	4.0

* p<0.05 **p<0.01

Conclusion:
Chloridazon caused clear maternal toxicity (food consumption, body weight and bodyweight development, piloerection) during treatment at the 250 mg/kg bw dose level and only very mild, transient effects at 50 mg/kg bw. A NOAEL for maternal toxicity has been established

at 10 mg/kg bw. There was no indication of malformations or embryo-/fetotoxicity at any dose level tested. Thus the following NOAELs were achieved:

NOAEL maternal toxicity:	10 mg/kg bw
NOAEL embryo-/fetotoxicity:	250 mg/kg bw
NOAEL malformations:	250 mg/kg bw

B.6.6.2.2 Rabbit

Report: Becker H. et al., 1987
Dose-finding embryotoxicity (including teratogenicity) study with chloridazon technical (ZNT-No. 86/99) in the rabbit
[redacted]
unpublished
BASF RegDoc# 1987/0412

GLP: No, not subject of GLP regulations

Guideline: Dose-finding study

Deviations: Not relevant

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Test substance was identified as follows:
Chloridazon technical, gray crystalline solid
Batch No.: K50/16A
Purity: 95.3 %
Test substance No.: 86/99
Test animals: Chinchilla rabbits (KFM: CHIN, Hybrids, SPF Quality)

Chloridazon was administered to three female Chinchilla rabbits (KFM: CHIN, Hybrids, SPF Quality) per dose level from day 6 to 18 after mating by gavage in 4 % aqueous carboxymethylcellulose sodium salt (CMC) preparation. Doses selected were 0 (control), 100, 250 and 500 mg chloridazon/kg bw in a constant volume of 4 mL/kg bw with daily adjustment to the actual body weight. The dosing scheme can be seen in Table B.6.6-13. As the test substance was prepared daily, no analytical control was performed in this range-finding study. Body weight and food consumption were monitored throughout the study. The animals were examined twice daily for mortality and clinical symptoms. All surviving animals were sacrificed on day 28 post coitum and the fetuses were delivered by cesarean section. Post mortem examinations, including gross macroscopic examinations of all internal organs. In addition the uterus content, position of the fetuses and number of corpora lutea were examined.

Table B.6.6-13: Dosing scheme - prenatal (gavage) toxicity range-finding study in Chinchilla rabbits

Group	Females mated	Dose volume (mL/kg)	Dose (mg/kg bw)
1 (control)	3	4	0 (aqueous CMC* only)
2	3	4	100
3	3	4	250
4	3	4	500

* Test substance prepared in 4 % aqueous CMC (carboxymethylcellulose sodium salt)

Findings:
Analyses for homogeneity and intended dosages were performed in the main study; they verified the nominal concentrations and confirmed the stability.
There were no clinical symptom's observed in the dams and there was no mortality. One of the control animals was not pregnant. Food consumption was decreased in a dose related manner in the mid and high dose animals. Bodyweight loss was noted at the high dose during the first 6 days of treatment and bodyweight recovery after treatment was not complete at study termination. The corrected body weight gain showed a slight loss (1.5 %) in all chloridazon-treated animals however a dose related effect could not be observed.
Necropsy findings in one high dose female (separate liver lobe covered with a membrane and of necrotic appearance) were not considered treatment related, as was the total embryonic resorption in one low dose animal. The numbers of corpora lutea, of implantation's, live fetuses as well as the pre-implantation loss was similar in all dose groups. A slight increase in the incidence of fetal resorptions was noted at the high dose.
No malformed or anomalous fetuses were observed in any group. The mean fetal body weight was similar in all groups. There was a difference in the sex ratio at the high dose (low percentage of males), however the group size of three animals was to small to allow a definitive conclusion if this was test substance related. There were no abnormal findings at visceral observation and crania examination after removing of the scalp.

Conclusion:
The dose ranges selected seem to be suitable for the main study.
As there were only three rabbits treated per dose group and only two rabbits in the controls that could be used for the assessment of prenatal toxicity, NOAELs for maternal and embryo-/fetotoxicity in rabbits should be derived from the main study [see 1987/0413 Becker H. 1987].

Report: Becker H., 1987
Embryotoxicity (including teratogenicity) study with chloridazon technical (ZNT-No. 86/99) in the rabbit
[redacted]
unpublished
BASF RegDoc# 1987/0413

GLP: Yes (laboratory certified by Eidgenössisches Departement des Inneren, Bern, Schweiz)

Guideline: OECD 414, EPA 83-3, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Test substance was identified as follows:
Chloridazon technical, gray crystalline solid
Batch No.: K50/16A
Purity: 95.3 %
Test substance No.: 86/99

Test animals: Chinchilla rabbits (KFM: CHIN, Hybrids, SPF Quality)

Chloridazon was administered to sixteen female Chinchilla rabbits (KFM: CHIN, Hybrids, SPF Quality) per test group from day 6 to 18 after mating by gavage in a 4 % aqueous carboxymethylcellulose sodium salt (CMC) preparation. Doses selected were 0 (control); 55; 165 and 495 mg chloridazon/kg bw in a constant volume of 4 mL/kg bw with daily adjustment to the actual body weight. The dosing scheme can be seen from Table B.6.6-14. The test substance was prepared daily.
Body weight and food consumption was monitored throughout the study. The animals were examined twice daily for mortality and clinical symptoms. All surviving animals were sacrificed on day 28 post coitum and the fetuses were delivered by cesarean section. Post mortem examinations, including gross macroscopic examinations of all internal organs. In addition the uterus content, position of the fetuses and number of corpora lutea were examined. Fetuses were sexed, dissected carefully and examined for internal and skeletal findings.

Table B.6.6-14: Dosing scheme of the prenatal (gavage) toxicity study in Chinchilla rabbits

Group number	Number of females mated	Dosage volume mL/kg/day	Dosage level mg/kg bw
1 (control)	16	4	0*
2	16	4	55
3	16	4	165
4	16	4	495

* Treated with the vehicle (4 % sodium carboxymethylcellulose sodium salt) only

Findings:

Analysis for homogeneity and intended dosages verified the nominal concentrations and confirmed the stability
Maternal data are compiled in Table B.6.6-15 and reproduction/fetal parameters in Table B.6.6-16. There were no mortalities or clinical findings that could be attributed to the test substance administration. One animal had to be sacrificed on day 7-post coitum due to a uterine prolaps, which was assessed not to be test substance related.
Food consumption was reduced at the high and to a lesser degree at the mid dose group during the dosing period. A compensatory increase was seen in these two groups during the post-treatment period (days 19-28 p.c.). A body weight loss was observed at the start of dosing in the high dose group and to a lesser degree at the mid dose group. Again a compensatory increase was noted after the dosing period. Corrected bodyweight gain was clearly affected at the high dose and to a smaller extend at the mid dose.

Table B.6.6-15: Prenatal toxicity (gavage) study in Chinchilla rabbits – Summary of maternal data

Dose (mg/kg bw)	0	55	165	495
Mated females on study	16	16	16	16
Pregnant females on study	13	15	15	15
Mortality of dams	0	0	0	0
Clinical symptoms	-	-	-	-
Food consumption (day 6-11 p.c. in g/animal/day and %)	190 100 %	204 107 %	182 96 %	149 78 %
Body weight gain (day 6-11 p.c. in g/animal/day and %)	100 + 3.4 %	123 + 4.1 %	46 + 1.5	-20 - 0.7 %
Corrected body weight gain (net weight change from day 6-28 in %)	+ 2.1	+ 2.2	+ 0.7	- 0.6

Reproduction parameters as assessed by pregnancy rate, number of total resorptions, corpora lutea implantations, live fetuses or pre-/postimplantation loss showed no adverse effect to the test substance administration.
Prenatal toxicity parameters, as assessed by sex ratios, fetal bodyweight, external and visceral examination as well as the examination of the head and skeletal development were not adversely affected by the chloridazon treatment. Cyst like dilatation of the cerebrum noted in 4 fetuses of chloridazon treated animals (1/84 at 55 mg/kg bw; 2/111 at 165 mg/kg bw; and 1/115 at 495 mg/kg bw) were also noted in historical control animals and are reported to be due to preparation artifacts according to Joosten, H. F. P. et al. [see 1981/10184, Joosten H. F. P. et al., 1981; Cysts in rabbit foetal brains, Arch. Toxicol. 47, 25-37, 1981].

Table B.6.6-16: Prenatal toxicity (gavage) study in Chinchilla rabbits – Summary of reproduction/fetal parameters

Dose (mg/kg bw)	0	55	165	495
Females mated	16	16	16	16
Pregnant dams	13	15	15	16
Dams used for calculation	13	12	15	15
Pregnant at cesarean section (%)	81	94	94	100
Dams with abortion	0	0	0	0
Dams with all resorptions	0	1	0	1
Female mortality	0	0	0	0
Corpora lutea/dam (mean)	7.8	8.0	8.9	9.4
Implantations/dam (mean)	6.8	7.5	8.1	8.3
Preimplantation loss/dam (mean)	1.0	0.5	0.8	1.1
Postimplantation loss (mean)	5.4	7.5	9.4	7.4
Total resorptions/dam (mean)	0.4	0.5	0.7	0.7
Early resorptions/dam (mean)	0.2	0.3	0.3	0.3
Late resorptions/dam (mean)	0.2	0.2	0.3	0.5
Dead fetuses	0	0	0	0
Live fetuses/dam (mean)	6.5	7.0	7.4	7.6
Fetal weights in g (mean)	34.3	34.1	35.5	34.6

Conclusion:
Chloridazon caused maternal toxicity at doses of 165 mg/kg bw and above in form of impairment of food consumption and bodyweight. No adverse effects on reproduction parameters and embryo-/fetal development - including the occurrence of malformations - could be noted even at the high dose, which showed clear maternal toxicity. The following NOAELs were derived from this study:

NOAEL maternal toxicity:	55 mg/kg bw
NOAEL embryo-/fetotoxicity:	495 mg/kg bw
NOAEL malformations:	495 mg/kg bw

B.6.7 Delayed neurotoxicity (Annex IIA 5.7)

Studies on delayed neurotoxicity potential of chloridazon were considered to be not relevant and were not conducted. It is noted that the structure of chloridazon is not similar or related to that of compounds known to be capable of inducing delayed neurotoxicity.

With regard to neurotoxicity no indications of an acute or subchronic neurotoxic effect could be derived from the toxicity studies performed with chloridazon. Chloridazon showed no clinical signs in any of the toxicity studies which could be attributed to neurotoxicity, and no indications on histopathological changes on the central or peripheral nerve system were seen in the short-term, long-term or reproductive/developmental studies in rats, mice and dogs.

B.6.8 Further toxicological studies (Annex IIA 5.8)

Conclusion for chloridazon metabolite B

Chloridazon metabolite B was demonstrated to be virtually non toxic after acute oral administration with a LD_{50} in rats of approx. 5000 mg/kg bw/d for males and > 5000 mg/kg bw/d for females.
Chloridazon metabolite B did not show mutagenic or genotoxic properties in three tests, covering the end points of point mutation and *in vitro* chromosome aberration.
The short-term toxicity of chloridazon metabolite B is characterised mainly by kidney and descending urinary tract toxicity. Increased drinking water consumption and altered urinalysis parameters are considered to be related to the kidney toxicity. Kidney and urinary tract toxicity was observed in several studies. Female rats were more affected than males. The second target organ was the liver, with weight increases, altered fat distribution and increased number and size of vacuoles. At very high dose levels food consumption and body weight gain were impaired.
In a 4-week study in rats a NOAEL of 90 mg/kg bw/d was obtained. Three 3-month studies were conducted in rats. In an early study in Sprague-Dawley rats a NOAEL of 86 mg/kg bw/d was obtained. However, as the batch of the test substance was not identified and no analytical data provided the study was not used for the establishment of the most relevant NOAEL. In the second 3-month study in Wistar rats the LOEL was 750 ppm. A NOAEL was not established in this study. In a follow up 3-month study a NOAEL of 200 ppm (15 mg/kg bw/d) was obtained. The overall NOAEL for the short term toxicity of chloridazon metabolite B was 15 mg/kg bw/d.
In a prenatal toxicity study in rats maternal toxicity (reduced body weight gain and hematurea indicating kidney toxicity) was observed at 120 mg/kg bw, the NOAEL being 60 mg/kg bw. These findings are consistent with the observations in the short term feeding studies in rats. There were no signs of developmental toxicity including no test substance related malformations up to the top dose level of 120 mg/kg bw.
In conclusion chloridazon metabolite B was virtually not toxic after acute oral administration, it was not mutagenic or genotoxic and demonstrated no developmental toxicity. In short term

feeding studies the target organs were liver and kidney. The overall NOAEL for the short term toxicity of chloridazon metabolite B was 15 mg/kg bw/d.

Conclusion for chloridazon metabolite B-1

An acute oral toxicity study with chloridazon metabolite B-1 (Reg. No. 035 375) revealed an LD_{50} of 1200 mg/kg bw.
Chloridazon metabolite B-1 was not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay and not mutagenic in the CHO/HPRT mutation assay. It was negative in the in vitro UDS assay using primary rat hepatocytes. Additionally, chloridazon metabolite B-1 had no in vivo chromosome-damaging (clastogenic) effect in bone marrow cells of Wistar rats.
The no observed adverse effect level in a 3-month dietary rat study was 50 mg/kg bw for males and females (highest dose tested).
In a prenatal developmental study in Wistar rats maternal toxicity was substantiated at 50 mg/kg bw (highest dose level). Signs of substance-induced prenatal developmental toxicity, but no indications for teratogenicity occurred exclusively at the high dose level, which can be interpreted as secondary effect due to maternal toxicity. At 2 and 10 mg/kg bw no substance-induced signs of embryo-/fetotoxicity were observed. The no observed adverse effect level (NOAEL) for maternal and prenatal developmental toxicity was 10 mg/kg bw.
In conclusion, chloridazon metabolite B-1 was moderately toxic after acute oral administration, it was not mutagenic nor genotoxic. The NOAEL for maternal and prenatal developmental toxicity was 10 mg/kg bw. The NOAEL for short-term toxicity of chloridazon metabolite B-1 was 50 mg/kg bw.

Conclusion of the pharmacology testing

The results of the pharmacology testing of chloridazon can be summarised as follows:
The effect of chloridazon on the vegetative nervous system in vitro is predominantly parasympathicomimetic. This is suggested by the acetylcholine like effects on the isolated ileum, which could be inhibited by atropine. There were no effects on the intravasal and cardiovascular systems.
Other test substance related changes were less specific, contradictory, observed at toxic dose levels or at very high in vitro concentrations only.
Chloridazon did not induce pathological changes to the EEC at any dose level. There was a dose related prolongation of the waking phase in the EEC without stimulation of motor activity. These changes may be attributable to a cholinergic effect of the test substance.

Table B.6.8-1: Summary results from toxicity studies with chloridazon metabolite B and B1

Institute/year/ Reference	Study type/species/ dose levels	Results / Comments	NOAEL
Chloridazon metabolite B			
[1998/10413; Kuehlem C., Hellwig J.; 1998] [redacted]	Acute oral LD_{50} Wistar rats 5000 mg/kg bw	LD_{50} males Approx. 5000 mg/kg bw LD_{50} females > 5000 mg/kg bw	
[1977/0155; Leuschner F. et al.; 1977a] [redacted]	4 week dietary study Sprague Dawley rat Target dose levels: 0; 30; 90 and 270 mg/kg bw	270 mg/kg bw: Slightly higher relative kidney weights in females. Increased dysplasia of mucosa of urinary bladder. Chronic interstitial nephritis in two rats. Target organs: kidney and urinary bladder	90 mg/kg bw
[1996/10817; Mellert W., Hildebrand B.; 1996a] [redacted]	3 month dietary study Wistar rats 0; 750; 2250 and 4500 ppm	4500 ppm: Smeared fur, reduced food consumption, increased drinking water consumption, slightly reduced body weight, decreased red blood cell values, altered urinalysis parameters. Kidney and ureter concretions, foci and retractions in the kidney and urothelial hyperplasia in ureter in females. Diffuse urothelial hyperplasia in the urinary bladder and renal pelvis in both sexes. 4500 and 2250, 750 ppm: Increased liver weights (considered as an adverse effect) in males and females. Altered fat distribution, increased number and size of vacuoles in liver of 2250/4500 ppm males and 4500 ppm females 750 ppm: increased absolute and relative liver weights in females Target organs: kidney and descending urinary organs and liver	NOAEL < 750 ppm 50 mg/kg bw males 60 mg/kg bw females

Institute/year/ Reference	Study type/species/ dose levels	Results / Comments	NOAEL
[1996/10818; Mellert W., Hildebrand B.; 1996b] [redacted]	3 month dietary study Wistar rats 0; 200 and 400 ppm	400 ppm: statistically significant increase of absolute and relative kidney weights in males and relative liver weights in females	NOAEL = 200 ppm 15 mg/kg bw males 17 mg/kg bw females
[1977/0156; Leuschner F. et al.; 1977b] [redacted]	3 month dietary study Sprague Dawley rat Target dose levels: 0; 30; 90 and 270 mg/kg bw	Study not acceptable: 270 mg/kg bw: Slight sedation. Reduced body weight, slightly increased relative kidney weights in females, delation of renal pelvis and urinary bladder in individual females, epithelial dysplasia of renal pelvis including scar formation and increased epithelial dysplasia of the mucosa of urinary bladder predominantly in females Target organs: kindney and urinary bladder	NOAEL: 86 mg/kg bw
[1992/10844; Engelhardt G., Hoffmann H. D.; 1992] BASF	Ames test TA 1535, TA 100, TA 1537 and TA 98 Standard plate assay and preincubation test with and without S9 mix	Negative	
[1999/11478; Wollny H. E., Arenz M.; 1999] RCC [1999/1003077; Wollny H. E.; 1999] RCC	*In vitro* mammalian cell HPRT test Chinese hamster V79 cells with and without S9 mix	Negative	
[1993/10075; Engelhardt G., Hoffmann H. D.; 1993] BASF	*In vitro* cytogenetics human lymphocytes with and without S9 mix	Negative	
[1997/10597; Hellwig J., Hildebrand B.; 1997] [redacted]	Prenatal toxicity Wistar rats 0, 20, 60 and 120 mg/kg bw (gavage) from day 6 through 15 post coitum	120 mg/kg bw: Impaired body weight gain and hematurea in dams Fetuses: No test substance related effects	Maternal toxicity NOAEL = 60 mg/kg bw Developmental toxicity: NOAEL =120 mg/kg bw

Institute/year/ Reference	Study type/species/ dose levels	Results / Comments	NOAEL
Chloridazon metabolite B-1			
[1999/10903; Manciaux X.; 1999] [redacted]	acute oral LD_{50} Wistar rats 200; 1000 and 5000 mg/kg bw	LD_{50} 1200 (615 - 2340) mg/kg bw	
[2001/1014868; Mellert W. et al., 2001] [redacted]	3 month dietary study Wistar rats 0; 2; 10 and 50 mg/kg bw	no test substance related adverse effects	NOAEL = 50 mg/kg bw
[1999/11415; Engelhardt G., Hoffmann H. D.; 1999] BASF	Ames test TA 1535, TA 100, TA 1537, TA 98 and *E.coli* WP2 uvrA Standard plate assay and preincubation test with and without S9 mix	Negative	
[2000/1010207; Engelhardt G., Hoffmann H. D.; 2000a] BASF	HPRT test CHO cells with and without S9 mix	Negative	
[2000/1018811; Engelhardt G., Hoffmann H. D.; 2000b] BASF	Unscheduled DNA synthesis *in vitro* primary rat hepatozytes	Negative	
[2000/1018864; Engelhardt G., Hoffmann H. D.; 2000c] BASF	*In vivo* bone marrow chromosome analysis in Wistar rats; 250, 500 and 1000 mg/kg bw (single oral administration)	Negative	
[2002/1000102; Kaspers U. et al.; 2002] BASF	prenatal toxicity Wistar rats 0; 2; 10 and 50 mg/kg bw (gavage) from day 6 through 19 post coitum	50 mg/kg bw: Dams: impaired absolute and corrected body weight gain, decreased mean gravid uterus weight, effects on clinical parameters (metabolic process liver, mild polycythemia); No influence on gestational parameters; Fetuses: reduced mean fetal body weights and increased skeletal variations due to fetal growth retardations and maternal stress; no indications for teratogenicity; 10 and 2 mg/kg bw: no test substance related effects on dams, gestational parameters or fetuses	Maternal toxicity NOAEL = 10 mg/kg bw Developmental toxicity NOAEL = 10 mg/kg bw

B.6.8.1 Toxicity studies of metabolites

B.6.8.1.1 BH 119-Metabolite B

Report: Kuehlem C., Hellwig J., 1998
Reg. Nr. 014 456 - Acute oral toxicity in rats
[redacted]
unpublished
BASF RegDoc# 1998/10413

GLP: Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 401, EEC 92/69, EPA 81-1

Deviations: None (However, raw data on chemical analysis missing)

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
BH 119-Metabolite B (chloridazon metabolite B; Reg. No. 014 456), brown powder
Batch: 41-216
Purity: 99.8 %
Test Substance No.: 98/22-1
Test animals: Wistar rats

Five male and female Wistar rats per dose group were treated with chloridazon metabolite B in 0.5 % aqueous Tylose CB 30.000 by gavage at a dose level of 5,000 mg/kg bw. 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:
Chemical analysis confirmed homogeneity, stability and correctness of the concentration of the test substance preparation. In addition, the storage stability of chloridazon metabolite B was verified.
In males 2 out of five animals died, in females 1 out of five. Mortalities in males occurred at 2 and 4 days after the administration. In females the single mortality occurred at 5 days after dosing.
Clinical symptoms recorded in males and females were impaired or poor general state, dyspnea, apathy, staggering, erythema, piloerection, smeared fur, exsiccosis, ataxia, tremor and red colored urine. They can be considered as non specific. In males the symptoms were observed during the first six days, in females during the first seven days. The expected body weight gain was generally observed during the study, with the exception of one female, which showed stagnation during the first week. There were no gross pathological findings in animals, which died, or in the surviving rats.

Conclusion:
The LD_{50} in Wistar rats was:

Males	approx.	5000 mg/kg body weight
Females		> 5000 mg/kg body weight

Report: Leuschner F. et al., 1977(a)
Oral toxicity of metabolite B, assay 98 %, in the Sprague-Dawley rat - repeated dosage over 4 weeks
[redacted]
unpublished
BASF RegDoc# 1977/0155

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

Guideline: Range-finding study, comparable to OECD 407

Deviations: Total cholesterol in clinical biochemistry missing

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
BH 119-metabolite B (chloridazon metabolite B, 4-amino-5-chloropyridazone(6))
Purity: 98 %
Batch: not indicated
Test animals: Sprague-Dawley rats

Chloridazon metabolite B was administered to 20 male and 20 female Sprague-Dawley rats per dose group over a period of 4 weeks via the feed. The dose levels were weekly adapted to the body weight and food consumption of the animals to achieve a test substance intake of 0; 30; 90 and 270 mg/kg bw.
The animals were examined each day for clinical symptoms and mortalities. Food consumption was determined daily. Body weight was recorded at weekly intervals.
Haematology was investigated before the start and at the end of the study. It consisted of: haemoglobin, erythrocyte and leucocyte count, differential blood count, haematocrit, platelets, reticulocytes, prothrombin time and blood clotting time.
Clinical-chemistry was performed before the start and at the end of the study. It consisted of: Alanine aminotransferase, glucose, blood urea, alkaline phosphatase, aspartate aminotransferase, total bilirubin, total protein, sodium potassium, calcium, chloride and uric acid.
Urinalysis was also performed before the start and at the end of the study.
An ophthalmological examination was carried out prior to sacrifice.
Terminal investigations consisted of organ weight determination, gross necropsy followed by a full histopathological evaluation.

Findings:
There are no data on the stability of the test substance. The stability of the test substance in the food has been demonstrated in other studies. There are no data on the homogeneity and the correctness of the dietary concentrations.
The target test substance intake of 0; 30; 90 and 270 mg/kg bw was achieved.
There were no mortalities or signs of clinical toxicity at any dose level.
There were no test substance related effects on body weight, food consumption, haematology, clinical chemistry, urinalysis or ophthalmoscopy at any dose level.
Relative kidney weight was slightly higher in high dose females.
There were no test substance related macroscopic changes noted.
Histopathological examinations revealed the following changes at the high dose level (270 mg/kg bw) only:
Increased epithelial dysplasia of the mucosa of the urinary bladder, occasionally with inflammation was seen. In the kidney two rats showed a chronic intestitial nephritis and one rat exhibited a purulent ascending pyelonephritis.
There were no other test substance related changes observed.

Conclusion:
This study identified the kidney and bladder as target organs.
The NOAEL for this study was 90 mg/kg bw.

Report: Mellert W., Hildebrand B., 1996(a)
Study of the oral toxicity of Reg. No. 14 456 (chloridazon-metabolite B) in Wistar rats. Administration via the diet over 3 months
[redacted]
unpublished
BASF RegDoc# 1996/10817

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

Guideline: OECD 408, EEC 87/302, EPA 82-1, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
BH 119-metabolite B (chloridazon metabolite B; Reg. No. 14 456)
Batch No. L 45/253
Purity: 98.2 %
Test substance No.: 90/435
Test animals: Wistar rats

Chloridazon metabolite B was administered to 10 male and 10 female Wistar rats per group at dietary dose levels of 0; 750; 2250 and 4500 ppm over a period of 3 months.
The animals were observed daily 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. As drinking water consumption was found to be increased in the high dose females, this parameter was determined in all groups during the last 4 weeks of treatment.
Prior to the beginning and towards the end of the study ophthalmological examinations were performed on all animals of the control and high dose group. Clinical-chemical and haematological examinations as well as urinalysis were performed on all animals after approximately 1.5 month of test substance administration and at the end of the administration period.
All animals were subjected to a full macroscopic and histopathological examination. Weights of selected organs were determined.

Findings:
The stability of the test substance was analytically proven. 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.6.8-2: Test substance intake

Dose group (ppm)	Test substance intake Males in mg/kg bw	Test substance intake Females in mg/kg bw
750	50	60
2250	155	180
4500	310	370

There were no mortalities in any of the groups. There were also no ophthalmological findings in any group.
At the high dose level the following findings were obtained:
Smeared fur in two females.
Reduced food consumption during the first two weeks of treatment in females.
Increased drinking water consumption in females.
Body weights were slightly reduced (approximately 6 %) in both sexes.
Body weight gain, calculated for the purpose of this assessment was also reduced at the high dose level. Male control body weight gain over the entire study period was 271.4 g, in high dose males it was 243.8 g, i.e. a reduction of 11 %. Female control body weight gain over the entire study period was 107.1 g, in high dose females it was 90.4 g, i.e. a reduction of 16 %.
The following haematological parameters were affected at the high dose level only:
Decreased red blood cells, hemoglobin and hematocrit in both sexes. Increased polychromasia and anisocytosis in both sexes. Increased polymorphonuclear neutrophils in females.
Clinical-chemical examinations revealed reduced alanine aminotransferase and alkaline phosphatase values as shown in the tables below:

Table B.6.8-3: Alanine aminotransferase and alkaline phosphatase values in rats at study week 44

Dose group (ppm)	Alanine aminotransferase		Alkaline phosphatase	
	Males	Females	Males	Females
0	1.04	1.04	5.92	5.58
750	0.97	0.97	5.87	4.73
2250	0.90**	0.91	5.51	4.86
4500	0.84**	0.92	5.34	4.45

** $P < 0.01$

Table B.6.8-4: Alanine aminotransferase and alkaline phosphatase values in rats at study week 87

Dose group (ppm)	Alanine aminotransferase		Alkaline phosphatase	
	Males	Females	Males	Females
0	1.02	0.90	4.64	4.32
750	0.85**	0.91	4.59	3.74
2250	0.84**	0.76**	4.37	3.48*
4500	0.72**	0.88	3.64**	3.15**

* P < 0.05, ** P < 0.01

The liver enzyme alanine aminotransferase is generally determined as a parameter for liver toxicity in case of an increase. A decrease of this enzyme is not associated with any pathological changes and is not considered to represent an adverse effect.
Urinalysis revealed several test substance related findings at high dose level only:
Increased calcium phosphate crystals and turbidity in the urine of both sexes.
Increased urinary volume and decreased specific urinary gravity in females.
Also in high dose females only an increase in blood, erythrocytes, renal tubular epithelial cells and transitional epithelial cells were found in the urine.
Organ weight analysis revealed an increase of relative and absolute kidney weight in high dose females.
Liver weights were also increased as shown in the table below:

Table B.6.8-5: Absolute and relative liver weight in rats

Dose group (ppm)	Absolute liver weights (g)		Relative liver weights	
	Males	Females	Males	Females
0	16.268	7.234	3.423	2.846
750	16.753	8.201*	3.62	3.068* #
2250	18.719	8.932**	3.953**	3.312**
4500	19.528*	9.281**	4.381**	3.846**

* P < 0.05, ** P < 0.01, # : within historical control range

For the 2250 ppm and 4500 ppm animals there was a statistically significant increase of relative and absolute liver weights. For 750 ppm females the observed increase in relative and absolute liver weights was also statistically significant.
Gross and histopathological investigations revealed the following changes:
Yellow-white doughy concretions in the ureter and renal pelvis as well as foci and retractions in the kidneys of high dose females. Diffuse urothelial hyperplasia in the urinary bladder and renal pelvis in both sexes at the high dose level. Diffuse urothelial hyperplasia was also observed in the ureter of high dose females.
Altered fat distribution with an increased number and size of vacuoles was observed in the liver of high dose males and females as well as in the 2250 ppm group males.

Conclusion:
Target organs were liver, kidney and descending urinary organs.
The only test substance related parameters at the low dose (750 ppm) were an increase in liver weights in females and a reduction in alanine aminotransferase in males. This liver enzyme is generally determined as a parameter for liver toxicity in case of an increase. Although a decrease of this enzyme is not associated with pathological changes and is not considered to represent an adverse effect the dose of 750 ppm is based on the increased liver weights con-

sidered as a LOAEL. To provide security for this assessment a further study with lower dose levels was performed. A NOAEL was not established in this study.

Report: Mellert W., Hildebrand B., 1996(b)
Study of the oral toxicity of Reg. No. 14 456 (chloridazon-metabolite B) in Wistar rats. Administration in the diet for 3 months
(Supplementary study to 31S0435/90031)
[redacted]
unpublished
BASF RegDoc# 1996/10818

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

Guideline: OECD 408, EEC 87/302, EPA 82-1, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Test substance was identified as follows:
BH 119-metabolite B (chloridazon metabolite B; Reg. No. 14 456)
Batch No.: L 45/253
Purity: 98.2 %
Test substance No.: 90/435
Test animals: Wistar rats

To confirm the assessment in the previous study that there is no adverse effect with chloridazon metabolite B a further short-term study was performed in rats.
Chloridazon metabolite B was administered to 10 male and 10 female Wistar rats per group at dietary dose levels of 0; 200 and 400 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.
Prior to the beginning and towards the end of the study ophthalmological examinations were performed on all animals of the control and high dose group. Clinical-chemical and haematological examinations as well as urinalysis were performed on all animals after approximately 1.5 month of test substance administration and at the end of the administration period.
All animals were subjected to a full macroscopic and histopathological examination. Weights of selected organs were determined.

Findings:
The stability of the test substance was analytically proven. 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.6.8-6: Test substance intake

Dose Group (ppm)	Test substance intake Males in mg/kg bw	Test substance intake Females in mg/kg bw
200	15	17
400	29	34

There were no mortalities or clinical signs of toxicity in any of the groups.
There were no effects on food consumption, body weight, ophthalmological or on the laboratory investigations.
Organ weight analysis revealed statistically significant increases in kidney and liver weight as shown in the tables below:

Table B.6.8-7: Absolute and relative liver weight in rats

Dose group (ppm)	Absolute liver weights (g)		Relative liver weights	
	Males	Females	Males	Females
0	14.583	7.31	3.204	2.772
200	13.703	7.682	3.129	2.987* #
400	15.726	7.85	3.402* #	3.08** #

* P < 0.05, ** P < 0.01, # within historical control range

Table B.6.8-8: Absolute and relative kidney weight in rats

Dose group (ppm)	Absolute kidney weights (g)		Relative kidney weights	
	Males	Females	Males	Females
0	2.847	2.022	0.627	0.768
200	2.985	1.989	0.683*	0.776
400	3.176**	2.007	0.688**	0.787

* P < 0.05, ** P < 0.01

The absolute kidney weight in males is increased in the high dose (400 ppm), the relative kidney weight in both dose groups. Liver weight changes were observed only in the relative weights in the high dose group in males and in both dose groups in females.
Gross and histopathological investigations did not reveal any test substance related changes.

Discussion:
Considering the absolute weight changes in the high dose group, the dose dependent weight changes in both dose groups and the lack of histopathological changes 400 ppm are considered as LOAEL and 200 ppm as NOEAL.

Conclusion:
Based on the statistically significant kidney and liver weight changes 400 ppm is considered as the LOAEL. The NOAEL in this study was 200 ppm or 15 mg/kg bw in males and 17 mg/kg bw in females.