Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification

bentazone (ISO), 3-isopropyl-2,1,3-benzothiadiazine-4-one-2,2-dioxide

EC Number: **246-585-8**

CAS Number: **25057-89-0**

Index Number: **613-012-00-1**

Contact details for dossier submitter:

RIVM, Bureau REACH

PO Box 1, 3720 BA Bilthoven, The Netherlands

Bureau-reach@rivm.nl

Version number: 2.0 **Date: 1 October, 2019**

CONTENTS

1. PHYSICAL HAZARDS

Not evaluated in this dossier

2. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

A series of studies has been performed on rats, mice and rabbits to determine the kinetics of bentazone. The metabolic pathway of bentazone was elucidated in these species following oral (rat and rabbit) or intravenous administration (mouse).

2.1.1 Study 1 – oral, rat

Anonymous 1987 (Doc. No. 87/0429)

The biokinetics and metabolism of [^{14}C]-bentazone in rats; BASF Reg. Doc. No. 87/0429

Testing facility: date of experimental work completed: 6 August 1987

Remark: The metabolism part of this study is described and evaluated in section 2.1.8.

Previous evaluation: in original DAR

Material and methods

Test method: according to EPA/FIFRA, Subdivision F, 85-1. The study is in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.

Deviations: None.

GLP: When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP.

Acceptance: The study is considered acceptable.

Objective: The pharmacokinetics and metabolism of [^{14}C]-bentazone in the rat were investigated. The experiments were designed to obtain data on the absorption, distribution, rates and routes of excretion and biotransformation of [^{14}C] -bentazone.

The effects of different dose levels and repeat dose administration on these parameters were also investigated. The biokinetics of the sodium salt of bentazone was compared with that of bentazone ("free acid"). In addition, tissue accumulation has been assessed after seven daily oral doses. A preliminary study was performed to determine whether significant amounts of [^{14}C] would be eliminated in the expired air.

Test system:

Test materials:

1. [U-phenyl-^{14}C]-bentazone, radiochemical purity > 97%; suspension in 1.5% CMC;

2. [U-phenyl-^{14}C]-bentazone sodium salt, radiochemical purity > 97%; solution in water (oral dose) or in 0.9% NaCl-solution (intravenous dose).

Male and female CD rats (source: Charles River, Margate, Kent, England) were used in this study. The animals received standardized diet and water ad libitum. For the excretion studies, rats were placed in metabolism units which allowed separate collection of urine and feces, and in the case of the preliminary phase, of expired air.

The experimental design is shown in the following table:

Table 2.1.1-1: Dosing groups for pharmacokinetic studies of [U-phenyl-^{14}C] bentazone[1]

Group No.	Test Group	Route	[^{14}C]-bentazone[2] mean (range) in (mg/kg bw)	Number/sex	Remarks
Preliminary study:					
1	preliminary	oral	198 (190 - 200)	2	-
Main study:					
2	low dose	oral	3.8 (3.6 - 4.0)	5	-
3	high dose	oral	205 (200 - 210)	5	-
4	low dose with pre-treatment	oral	3.6 (3.3 - 3.7)	5	pre-treatment with non-radioactive bentazone once a day for 14 days, followed by a single oral dose of [^{14}C]-bentazone
5	low dose	i.v.	4.1 (3.9 - 4.3)	5	sodium salt
Bile excretion study:					
6	low dose	oral	3.6 (3.3 - 3.9)	3	Rats with cannulated bile ducts were used.
7	high dose	oral	195 (180 - 210)	3	Rats with cannulated bile ducts were used.
Plasma level study:					
8	low dose	oral	3.6 (3.6 - 3.7)	5	-
9	high dose	oral	197 (190 - 200)	5	-
10	low dose	oral	4.1 (3.9 - 4.2)	5	-
11	low dose	i.v.	4.5 (4.3 - 4.7)	5	-
Tissue residues study:					
12	low dose	oral	4.0 (3.3 - 4.4)	5 (quantitative analysis); 6 males (qualitative)	7 daily doses given; animals were serially sacrificed (0.5 - 120 hours) to assess tissue residues quantitatively or by whole-body autoradio-graphy (qualitatively).

[1] Bentazone applied as free acid, unless specified as sodium salt.

[2] The doses of [^{14}C]-bentazone administered to individual rats were given for all groups.

Statistics: Statistical analysis was not performed.

Results

Tissue distribution and excretion studies

Preliminary experiment:

Results from the preliminary experiment indicated that the major part of the administered radioactivity was excreted in the urine including cagewash by males (95.3%) and females (92.3%) during 5 days. Most of this radioactivity was found in the 0-24 hour urine already. By 120 hours post dosing, less than 0.03% of the total dose was eliminated in the expired air in both sexes.

Single intravenous low dose:

Five days following administration of a single intravenous dose of [U-phenyl-^{14}C]-bentazone, sodium salt (mean 4.1 mg/kg bw, adjusted as bentazone free acid) to rats, total recovery had reached approximately 95.4% of the radioactivity in males and 90.2% in females (see Table 2.1.1-2). Elimination in the urine accounted for 93.87% of the dose in males and 88.96% in females; these values may be incremented by about 0.4% of the dose if radioactivity detected in the cage wash is included. Most (91.5% and 85.8% of the dose in males and females, respectively) of renal elimination was complete within the first 24 hours after dosing. Elimination in the feces accounted for about 1.18% of the dose in males and 0.51% of the dose in females. At sacrifice, total radioactive residue in the carcass amounted to 0.32% of the dose in females. Mean residues in kidneys amounted to 0.019 µg/g and 0.026 µg/g in males and females, respectively; and in uterus to 0.002 µg/g. Residues in all other tissues (see Table 2.1.1-3) were at or below the limit of measurement (2 x background radioactivity).

Single low oral dose:

Five days following administration of a single oral dose of [^{14}C]-bentazone, free acid (mean 3.8 mg/kg bw in rats, mean recovery was approximately 91.95% of the dose in males and 90.05% of the dose in females (Table 2.1.1.-2) . Elimination in the urine accounted for 90.0% of the dose in males and 88.6% in females when cagewash was included. 86.7% and 83.7% of the dose totally eliminated via urine was excreted within the first 24 hours after dosing already in males and females, respectively. Excretion in the feces accounted for about 1.50% of the dose in males and 0.76% of the dose in females. At sacrifice, total radioactive residue in the carcass amounted to 0.48% of the dose in males and to 0.69% in females. Mean values for all tissues were at or below the limit of detection (Table 2.1.1-3).

Table 2.1.1-2: Recovery[1] of radioactivity in tissues and excreta of rats 120 hours after a dosing with [phenyl-^{14}C]-bentazone

Percent of radioactive dose recovered								
	i.v.[2] 4.1 mg/kg bw		**oral 3.8 mg/kg bw**		**oral 205 mg/kg bw**		**oral pre-treatment plus 3.6**	
Matrix	**M**	**P[3]**	**M**	**F**	**M**	**F[3]**	**M**	**F**
Urine	93.87	88.96	89.48	88.13	94.32	93.03	95.86	90.49
Feces	1.18	0.51	1.50	0.76	2.27	2.00	0.92	1.44
Cage wash	0.35	0.41	0.49	0.47	0.30	0.58	0.03	0.07
Carcass	4	0.32	0.48	0.69	0.24	0.17	N.D.[5]	0.50
Total:	**95.40**	**90.20**	**91.95**	**90.05**	**97.13**	**95.78**	**96.81**	**92.50**

[1] Data from pages 39 - 42 of the study report. Urine calculated by subtracting cage wash values from reported urine + cage wash values.

[2] The four dosage groups depicted in the dosage columns of this Table correspond to Groups 2 - 5 in Table 2.1.1-1.

[3] Data obtained from four rats only.

[4] No data (<0.2%).

[5] N.D. - Not detected.

Table 2.1.1-3: Average distribution of radioactivity in rat tissues/organs 120 hours after dosing with [U-phenyl-^{14}C]-bentazone[1]

Mean total residue (µg/g)								
Matrix	**i.v.[2] 4.1 mg/kg bw**		**oral 3.8 mg/kg bw**		**oral 205 mg/kg bw**		**pre-treatment plus 3.6 mg/kg bw**	
	M	**F**	**M**	**F**	**M**	**F[3]**	**M**	**F**
Adrenals	<0.15	<0.083	<0.100	<0.0470	<5.40	<3.0	<0.077	<0.058
Bone marrow	<0.084	<0.170	<0.031	<0.570	<3.20	<4.0	<0.026	<0.065
Brain	<0.011	<0.012	<0.0062	<0.0062	<0.40	<0.345	<0.007	<0.072
Fat	<0.023	<0.020	<0.0120	<0.0130	<1.30	<0.730	<0.013	<0.014
G. I. tract	<0.011	<0.012	<0.0072	<0.0084	<0.33	<0.307	<0.0072	<0.0076
Heart	<0.012	<0.012	<0.0078	<0.0084	<0.42	<0.407	<0.0072	<0.0074
Kidneys	0.019	0.026	<0.0070	<0.0070	<0.42	<0.359	<0.007	<0.007
Liver	<0.012	<0.015	<0.0066	<0.0062	<0.41	<0.347	<0.007	<0.007
Lungs	<0.012	<0.012	<0.0074	<0.0084	0.42	<0.368	<0.007	<0.0074
Muscle	<0.019	<0.02	<0.0070	<0.0064	<0.42	<0.373	<0.014	<0.014
Ovaries	N. A.[4]	<0.032	N. A.	<0.0230	N. A.	<1.05	N. A.	<0.018
Pancreas	<0.012	<0.013	<0.0070	<0.0082	<0.47	<0.406	<0.0068	<0.0074
Plasma	<0.005	<0.005	<0.0032	<0.0030	<0.18	<0.179	<0.003	<0.003
Spleen	0.0098	<0.015	<0.0060	<0.0072	<0.47	<0.458	<0.006	<0.008
Testes	<0.012	N. A.	<0.0076	N. A.	<0.43	N. S.	<0.007	N. A.
Thyroid	0.57	<0.57	<0.3400	<0.3000	<23.00	<19.1	<0.21	<0.24
Uterus	N. A.	0.002	N. A.	<0.0090	N. A.	<0.558	N. A.	<0.01
Whole-Blood	<0.011	<0.011	<0.0068	<0.0066	<0.354	<0.508	0.0062	<0.007

[1] Data from Table 18, p. 55 (i.v. group); Table 15, p. 52 (low dose group); Table 16, p. 53 (high dose group) and Table 17, p. 54 (multiple dose pre-treatment) of the study report.

[2] The four dosage groups depicted in the dosage columns of this Table, correspond to Groups 2 - 5 of Table 2.1.1-1.

[3] Values were recalculated using individual animal data from Table 15 of appendix 4 (p. 121) of the study report. Rats 27 - 30 were included in the calculation, rat 26 was omitted due to significantly deviant values for some tissues.
* N. A. = not applicable.

Single high oral dose:

Five days following administration of a single oral dose of [^{14}C]-bentazone, free acid (mean 205 mg/kg bw) to rats, approximately 97.13% of the dose was eliminated in males and 95.78% in females (see Table 2.1.1-2). Renal excretion accounted for 94.32% in males and 93.03% in females; these values may be incremented by about 0.3% - 0.6% of the dose if radioactivity recovered in the cage wash is included. Most (92.0% and 91.0% of the dose in males and females, respectively) of the elimination in urine was complete within the first 24 hours after dosing. Elimination in the feces accounted for about 2.27% of the dose in males and 2.0% in females. At sacrifice, total radioactive residue in the carcass amounted to 0.24% of the dose in males and to

0.17% of the dose in females. Mean tissue residue values (summarized in Table 2.1.1-3) were all at or below the limit of measurement.

Single low oral dose with pre-conditioning:
Five days following administration of a single oral dose of [^{14}C]-bentazone, free acid (approximately 3.6 mg/kg bw) to rats, preceded by single daily oral doses of nonradioactive bentazone, free acid (nominally 4 mg/kg bw) for 14 days, approximately 96.81% of the radioactivity had been recovered from males and 92.50% from females (Table 2.1.1-2). Elimination in the urine accounted for 95.86% of the dose in males and 90.49% in females; these values may be incremented by about 0.03% - 0.07% of the dose if radioactivity recovered in the cage wash is included. Within the first 24 hours after dosing, 94.1% and 85.2% of the total excretion via urine was complete in males and females, respectively. Elimination in the feces accounted for about 0.92% of the dose in males and 1.44% of the dose in females with most of this in the 0-48 hour samples. At sacrifice, total radioactive residue in the carcass amounted to 0.5% of the dose in females and was undetectable in males. Residues in tissues were at or below the limit of measurement (see Table 2.1.1-3).

Additional studies on pharmacokinetics and tissue distribution:
As summarized in Table 2.1.1-1, further experiments were performed to investigate the pharmacokinetics of Bentazone. These trials included the examination of bile excretion using bile duct-cannulated rats, and elucidation of time course of plasma levels and tissue levels of radioactivity as well as autoradiography.

Bile excretion studies:
Excretion of radioactivity in bile was very limited only. At the high dose, biliary excretion amounted to 0.80% (females) or 1.84% (males) of the total dose. At the low dose level, means of 0.2% (females) and 1.3% (males) of the radioactivity administered were excreted in the bile. Biliary excretion was essentially complete by 24 hours post-dosing.

Time course of plasma radioactivity:
Peak of mean concentration of radioactivity in plasma after a single intravenous dose of the sodium salt was reached at 5 minutes (24 µg/ml). Following single oral doses, radioactivity in plasma reached a maximum by 15 or 30 minutes at the low dose levels (free acid or sodium salt, groups 8 and 10 of Table 2.1.1-1) and by one hour at the high dose (group 9). Determination of the area under the plasma radioactivity curve (AUC) per unit dose, revealed significantly higher values for the high dose groups compared to the low dose groups (Table 2.1.1-5) . This observation might suggest that a non-linear region in disposition is being reached at the higher dose. AUC values for females dosed with low oral doses were significantly smaller (nearly half) than AUC values for females dosed intravenously; corresponding values for males were not significantly different.

Table 2.1.1-4: Recovery of radioactivity in bile and excreta of bile duct-cannulated rats 48 hours after oral dosing with [U-phenyl-^{14}C]-bentazone

Percent of radioactive oral dose recovered with				
Matrix	**3.6 mg/kg bw**		**195 mg/kg bw**	
	M	**F**	**M**	**F**
Bile	1.31	0.24	1.84	0.80
Urine	89.48	78.20	88.64	83.68
Feces	3.00	1.52	1.99	1.71
Cage wash	0.44	1.29	1.12	1.15
Carcass	1.47	1.47	1.29	1.17
Total	**95.70**	**82.72**	**94.88**	**88.51**

Table 2.1.1-5: Mean areas under the plasma concentration-time curves (AUC) in rats dosed with [U-phenyl-^{14}C]-bentazone (free acid or sodium salt)

Test group (dose males, dose females, in mg/kg bw)	**Form of bentazone**	**Area Under the Curve (AUC) (µg x**	
		Males	**Females**
oral low (3.7, 3.6)	free acid	8.0 + 2.6	3.5 + 0.7
oral high (196, 198)	free acid	15.0 + 3.8	11.0 + 3.9
oral low (4.1, 4.0)	sodium salt	6.2 + 1.8	3.5 ± 1.1
i.v. low (4.5, 4.4)	sodium salt	6.0 + 2.0	6.8 ± 1.2

Time course of tissue levels of radioactivity:

Concentrations of radioactivity were highest at 0.5 hours after dosing in tissues obtained from pairs of rats sacrificed at different times after the last of seven low level oral doses of [^{l4}C]-bentazone. At this time, concentrations in most tissues were in the region from 0.1 µg/g to 5 µg/g. Higher concentrations were confined to the gastro-intestinal tract, kidney, thyroid and plasma (5 µ/g to 20 µg/g). At six hours, concentrations of radioactivity were generally in the region 0.05 µg/g to 1 µg/g apart from higher concentrations in the gastrointestinal tract, kidney, thyroid and plasma (0.5 µg/g to 5 µg/g). At 24 and 120 hours after the last dose, concentrations of radioactivity in all tissues examined were < 0.1 µg/g except in the thyroid (< 0.3 µg/g). A comparison of tissue distribution after a single dose and seven daily doses of [^{14}C]-bentazone showed no evidence of accumulation of radioactivity after repeated dosing. Whole-body autoradiography of treated rats in general confirmed the mentioned distribution pattern of radioactivity. However, the quality of the photocopies after the 24 hour time-point was too poor to be of great use for assessment. A certain affinity of the compound to the keratinized layer of squamous epithelium of the non-fundic mucosa of the stomach was detected since moderate levels of radioactivity were present there. In all tissues, steady disappearance of label with time became apparent.

Conclusion

The results of this study indicate a rapid absorption, distribution and primarily renal excretion of [^{14}C]-bentazone with no appreciable differences for the various dosing regimens applied. Biliary excretion was occurring to a limited degree only. There was no significant difference in the pharmacokinetics of orally administered bentazone free acid and its sodium salt. However, some sex-related differences were observed. Assessment of tissue residues and analysis of their time course did not reveal evidence for compound accumulation.

2.1.2 Study 2 – oral, rat

Anonymous 1975 (Doc. No. 75/0083)

A comparison of the bioavailability and metabolic fate of [^{14}C]-bentazone free acid and [^{14}C]-bentazone sodium salt in the rat, after oral administration; BASF Reg. Doc. No. 75/0083

Testing facility: experimental work completed: 1975

Previous evaluation: in original DAR

Material and methods

Test method: This study was performed prior to implementation of specific test guidelines. Thus, the major part of the study is not in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.

GLP: No. The study was performed prior to implementation of GLP guidelines.

Acceptance: The study is considered acceptable.

Objective: The study was performed to investigate whether there was any difference in the biological fate of bentazone applied in its free acid form compared with bentazone sodium salt. In addition, the excretion, retention and metabolic fate of the sodium salt has been studied and compared with previous results obtained with the free acid.

Test system: For measurement of radioactivity in plasma, two groups of 12 male Sprague-Dawley (CFY) rats (source: Anglia Laboratory Animals, Huntingdon, England) each were administered the test substances at a dose level of 4 mg/kg bw in a 25% (w/w) ethanol/water solution once a day by oral gavage. The first group received non-radioactive bentazone (batch no. not available) in its free acid form for a period of seven days. 24 hours after the seventh daily dose, each rat received a single dose of [^{14}C]-bentazone free acid (4 mg/kg bw; 1.49 mCi/mmole = 6.14 µCi/mg). The second group received non-radioactive bentazone sodium salt (batch no. not submitted) for a period of seven days. 24 hours after the seventh dose, each rat received [^{14}C]-bentazone sodium salt. From all animals, blood samples were removed from tail vein at intervals during 24 hours. For the excretion - retention study, three rats per sex were dosed by oral intubation with [^{14}C]-bentazone sodium salt (equivalent to 4 mg free acid/kg bw). Rats were housed in metabolism cages and received standardized diet and water ad libitum. Urine was collected at intervals of 0 - 8 hours and 8-24 hours

and then at intervals of 24 hours during five days. Feces were collected at 24-hour intervals for 48 hours and then as a single sample up to five days. After five days, the rats were killed.
Statistics: Statistical analysis was not performed.

Results

After oral administration of either [^{14}C]-bentazone free acid or the sodium salt to rats, no significant differences were detected in the maximal plasma concentrations of radioactivity (4.87 µg - 4.98 µg equivalents/ml) or in the plasma half-life of elimination (2.7 hours). No significant differences were found between the times taken for attainment of maximal concentrations of plasma radioactivity, although the mean time until the compound occurred in blood was shorter after dosing with the free acid (0.8 hours) than with the sodium salt (1.2 hours).
An oral dose of [^{14}C]-bentazone sodium salt was well absorbed and rapidly eliminated; during six hours after dosing means of 63% and 51% of the dose had been excreted in the urine by males and females respectively. After 24 hours, means of 90.0% and 91.3% had been excreted in the urine. At 120 hours, males had excreted a mean of 91.1% of the dose via urine; females had renally excreted 92.7% of total radioactivity. Fecal excretion during five days after dosing accounted for 1.8% and 0.95% of the dose in males and females; no radioactivity was detected in the carcass at this time.
The predominating compound eliminated in the urine was unchanged bentazone (81.4% and 85.3% of the administered dose in males and females, respectively). It was shown that the remaining radioactivity in the urine did not consist of glucuronides or sulfate conjugates.

Conclusion

The rate of absorption and the extent of bioavailability were similar after administration of either chemical form of bentazone tested. No significant differences were found in the pharmacokinetics or metabolism of bentazone sodium salt when compared with bentazone free acid. The biological behaviour of both chemical forms would be expected to be indistinguishable, therefore.

2.1.3 Study 3 – oral, rat

Anonymous 1971 (Doc. No. 71/0069)

The metabolism of bentazone in rats; BASF Reg. Doc. No. 71/0069
Testing facility: June 25, 1971; experimental work completed: 1971
Remark: Results of this study were published by Chasseaud LF et al. in 1972 (see reference list).
Previous evaluation: in original DAR

Material and methods

Test method: This study was performed prior to implementation of specific test guidelines. Thus, the major part of the study is not in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.
GLP: No. The study was performed prior to the implementation of GLP guidelines.
Acceptance: The study is scientifically valid. However, with respect to the year when the study was performed and some deficiencies in reporting, it is considered supplementary only.
Test system: A single oral dose of 0.8 mg (10 µCi)[^{14}C]-bentazone (labelled in the benzene ring, batch no. not submitted) per animal was administered by gavage as 50% aqueous ethanol solution to male and female Sprague-Dawley (CFY) rats (source: Carworth Europe). Absorption, distribution, excretion (including bile examination by means of biliary fistulae) and metabolism of [^{14}C]-bentazone were studied. During the metabolism study, rats were housed in metabolism cages and were supplied with food and water. Urine was collected at 3, 6, 12 and 24 hours after dosing and daily thereafter. Feces were collected daily. Expired gases were monitored for radioactivity. Rats were sacrificed after 4 days. Young male rats were given total oral doses of 2.4 mg [^{14}C]-bentazone, sacrificed at different intervals after dosing and subjected to whole-body autoradiography.
Statistics: Statistical analysis was not performed.

Results

An oral dose of [^{14}C]-bentazone was rapidly and almost quantitatively absorbed in rats. The radioactivity was rapidly excreted, mostly in the urine (approximately 91% within 24 hours); only traces were secreted into bile, although considerable individual differences were obvious (0 - 2.9%). During 4 days after dosing, less than 1% of the total dose was excreted in the feces and less than 0.02% in the expired air. Small amounts of radioactivity remained in the carcass after 4 days. Only 0.54% of the dose was recovered from the whole body at that time. Autoradiography elicited a rapid distribution of [^{14}C]-bentazone in rats. After one hour, levels of radioactivity were high in the stomach, liver, heart and kidney. Radioactivity was not detected in the brain or spinal cord. Thin layer chromatography showed that most of the radioactivity in the urine consisted of unchanged bentazone (84% of the radioactivity). Two minor unidentified metabolites were detected accounting for 2.3% and 0.8% of the radioactivity.

Conclusion

Bentazone was rapidly and readily absorbed, distributed and excreted in the rat. There was no evidence of tissue accumulation. The compound is primarily eliminated via urine and mainly as unchanged parent molecule.

2.1.4 Study 4 – oral, rat

Characteristics

Reference	:	Anonymous 2011a (Doc No 2011/1265806)	exposure	:	Single oral
type of study	:	Biokinetics study in rat.	doses	:	80 mg/kg bw BAS 351 H or 80 mg/kg bw BAS 351 H + 150 mg/kg bw Probenecid.
year of execution	:	2011	vehicle	:	1% carboxymethylcellulose
test substances	:	^{14}C-BAS 351 H (batch 210-2401), specific activity a.s. 5.32 MBq/mg, purity >95%; unlabelled bentazone (batch COD-001416), purity 100% ± 1.0%; bentazone-sodium (batch COD-001417), purity 91.9% ± 1.0%; probenecid (batch BCBC7346V), purity not reported.	GLP statement	:	Yes
Route	:	oral	guideline	:	According to OECD 417
Species	:	Rat, Wistar	acceptability	:	Acceptable
group size	:	Bentazone: five females Bentazone+probenecid: six females	Previous evaluation		Submitted for renewal (supplementary)

Study design

The aim of the study was to generate information on the influence of probenecid on plasmakinetics of radiolabeled ^{14}C-BAS 351 H (bentazone) in Wistar rats. Probenecid works by interfering with kidneys organic anionic transporter (OAT). It was of special interest if the administration of probenecid leads to higher internal dose levels of BAS 351 H (bentazone) in plasma (AUC) after oral dosing, giving the mechanism of active transport of bentazone in the kidney.

Two groups of female Wistar (Crl:WI (Han)) rats received a single oral target of 80 mg/kg bw ^{14}C-BAS 351 H (bentazone, calculated as bentazone acid) in 1% carboxymethylcellulose suspension in tap water by gavage either with or without intraperitoneal pre-treatment with probenecid, dissolved in 0.9% saline including 0.5% carboxymethylcellulose and 1% Cremophor EL, at 150 mg/kg bw. Blood was sampled at 0.5, 1, 2, 4, 8, 24, 48 and 72 hours post dosing and the plasma concentrations of radioactivity were determined. The plasma concentration versus time curves was used to calculate the $AUC_{0-\infty}$ and plasma half-lives

Results

The analytical investigations of ^{14}C-BAS 351 H demonstrated the stability, homogeneity and correctness of the nominal concentration of the test substance during the period of test substance administration. The test substance preparation was performed with the NA salt of BAS 351 H. An appropriate equivalent factor was applied to calculate the corresponding concentration for the acid form that was the relevant form for the definition of the target dose levels.

Table 2.1.4-1 Nominal and analytical concentrations compared to target concentrations

Dose group	Target concentration [mg/g]	Nominal concentration [mg/g]	Analytical results % of nominal concentration (Mean ± SD)
Bentazone	8.0 [1]; 9.5 [2]	9.52 [2]	98.2 ± 0.8 [2]

Dose group	Target concentration [mg/g]	Nominal concentration [mg/g]	Analytical results % of nominal concentration (Mean ± SD)
Probenezid	15.0	15.0	101.7 ± 1.2

[1] 351 H (bentazone) acid

[2] BAS 351 H (bentazone) Na salt

In female rats exposed to a mean actual dose of 85.09 mg/kg bw, the maximum plasma concentration of 223.79 µg Eq/g occurred 0.5 hours post-dosing (see Table 2.1.4-2). Afterwards, plasma levels declined to 0.1 µg Eq/g at sacrifice after 72 hours. The initial half-life was calculated to be 1.5 hours (see Table 2.1.4-3). Terminal half-life was 18.4 hours. The calculated area under the plasma concentration time curve (AUC) was 753 µg Eq*h/g.

In probenecid pre-treated female rats exposed to a mean single oral actual dose of 84.90 mg/kg bw ^{14}C- BAS 351 H (bentazone) acid equivalent, the maximum plasma concentration of 125.18 µg Eq/g occurred 4 hours post-dosing. Afterwards, plasma levels declined to 0.1 µg Eq/g at sacrifice after 72 hours. The initial half-life was calculated to be 2.7 hours. Terminal half-life was 25.9 hours. The calculated area under the plasma concentration time curve (AUC) was 1892 µg Eq*h/g.

Table 2.1.4-2 Mean plasma concentrations of radioactivity after single oral administration of ^{14}C- BAS 351 H (bentazone) at actual mean dose levels of 85.09 and 84.90 mg/kg bw to female Wistar rats with and without pretreatment with probenecid

Time [h]	^{14}C-BAS 351 H (Bentazone)	^{14}C-BAS 351 H (Bentazone) + Probenecid pretreatment
0.5	223.79	76.54
1	154.28	95.60
2	104.07	115.85
4	40.81	125.18
8	17.10	118.51
24	0.61	1.03
48	0.17	0.19
72	0.10	0.10

Table 2.1.4-3 Pharmacokinetic parameters of ^{14}C- BAS 351 H (Bentazone) in female Wistar rats with and without pretreatment with Probenecid

Actual dose	c_{max} [µg Eq/g]	T_{max} [h]	initial half life [h]	terminal half life [h]	$AUC_{0-\infty}$ [µg Eq *h/g]
85 mg/kg bw ^{14}C-BAS 351 H (Bentazone)	223.79	0.5	1.5	18.4	753
85 mg/kg bw ^{14}C-BAS 351 H (Bentazone) + Probenecid pretreatment	125.18	4.0	2.7	25.9	1892
Increase related to ^{14}C- BAS 351 H (Bentazone) group					2.51 X

Comparing the two experiments, an increase of the T_{Max}-value and a decrease in the maximum plasma concentration was observed when ^{14}C-BAS 351 H dosed female rats were pretreated with probenecid (Table 2.1.4-3). This might be due to a certain degree an inhibition of gastrointestinal resorption of ^{14}C-BAS 351 H by probenecid.

The AUC $_{0-\infty}$-value of ^{14}C-BAS 351 H, calculated from the plasma concentration versus time curves of the bentazone-group was 753 µg Eq x h/g, while that of the probenecid pre-treated group was calculated to be 1892 µg Eq x h/g, thereby showing an increase by a factor of 2.51. This increase of the $AUC_{0-\infty}$-value by probenecid pre-treatment is clearly indicative for an inhibition of renal excretion of BAS 351 H by probenecid.

The initial and terminal half-life were nearly doubled from 1.5 and 18.4 hours in the ^{14}C- BAS 351 H treated group to 2.7 and 25.9 hours in the Probenecid + ^{14}C- BAS 351 H treated group, respectively. Although being a rather insensitive indicator for excretory saturation phenomena, the data of initial and terminal half-lives in plasma further supported the conclusion that probenecid pre-treatment inhibits the renal excretion of BAS 351 H.

Conclusion

Probenecid pretreatment induced an increase of the AUC and of the initial as well as of the terminal half life in ^{14}C-BAS 351 H (bentazone) treated rats. These results clearly indicated inhibition of renal excretion of ^{14}C-BAS 351 H (bentazone) by probenecid already within the first 8 hours after administration. Since probenecid pretreatment blocks secretion via the Organic Anion transporter (OAT), it can be assumed that bentazone is actively secreted via the AOT.

2.1.5 Study 5 – oral, rat

Characteristics

Reference	:	Anonymous 2011b (Doc No. 2011/1262233)	exposure	:	Single oral
type of study	:	Biokinetics study in rat.	doses	:	Experiment 1: 40, 150, 500 mg/kg bw Experiment 2: 40, 80, 250 mg/kg bw
year of execution	:	2010-2011	vehicle	:	1% carboxymethylcellulose
test substances	:	^{14}C-BAS 351 H (batch 210-2301),	GLP statement	:	Yes

		specific activity a.s. 5.15MBq/mg, purity 96.5%; unlabelled bentazone (batch N 187), purity 99%;			
Route	:	oral	guideline	:	According to OECD 417
Species	:	Rat, Wistar	acceptability	:	Acceptable
group size	:	4 females/dose	Previous evalatuion		For renewal (supplementary)

Study design

The aim of the study was to generate information on the plasma kinetics of radiolabeled ^{14}C-BAS 351 H (bentazone). The study on the biokinetics was performed in order to elucidate a possible onset of saturation of renal excretion.

In experiment 1, the total radioactivity of ^{14}C-BAS 351 H (^{14}C-Bentazone) in blood and plasma (AUC) after a single oral dosing of 40, 150 and 500 mg/kg bw via gavage (calculated as acid form) was investigated.

In experiment 2, further dosing under the same conditions as in experiment 1 of 40, 80 and 250 mg/kg bw (calculated as acid form) was investigated in 8, 4 and 4 female Wistar rats, respectively.

Blood sample were taken from the retro orbital sinus under isoflurane anaesthesia at 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144 and 168 hours.

Results

The analytical investigations demonstrated the stability, homogeneity and correctness of the concentrations of ^{14}C-BAS 351 H for all performed experiment.

Table 2.1.5-1 Nominal and analytical concentrations compared to target concentrations

Dose group	Target concentration [mg/g]	Nominal concentration [mg/g]	Analytical results % of nominal concentration (Mean ± SD)
40 mg/kg bw	4.0	3.98	96.2 ± 0.6
40 mg/kg bw (2nd experiment)	4.0	4.10	102.8 ± 1.3
80 mg/kg bw	8.0	8.04	98.7 ± 4.0
150 mg/kg bw	15.0	14.9	91.8 ± 1.9
250 mg/kg bw	25.0	25.0	95.9 ± 4.9
500 mg/kg bw	50.0	49.34	95.4 ± 0.4

The target doses of 500, 250, 150, 80 and 40 (experiment 1 & 2) mg/kg bw ^{14}C- BAS 351 H resulted in maximum mean plasma concentrations of 600.22 µg Eq/g, 513.14 µg Eq/g, 353.77 µg Eq/g, 185.76 and 109.68 & 85.76 µg Eq/g, respectively. Afterwards plasma levels declined to 0.4 µg Eq/g in females of the

high dose group, as well as to 0.16, 0.17, 0.05 and 0.06 µg and 0.02 Eq/g in the 250, 150, 80 and 40 (exp.1 & 2) mg/kg bw groups, respectively (see Table 2.1.5-2).

Table 2.1.5-2 Mean plasma concentration of radioactivity after single oral administration of ^{14}C BAS 351 H at target dose levels of 500, 250, 150, 80 and 40 mg/kg bw to female Wistar rats

	Results expressed in µg Eq/g plasma					
Target dose[1]	**500 mg/kg bw**	**250 mg/kg bw**	**150 mg/kg bw**	**80 mg/kg bw**	**40 mg/kg bw** (exp. 1)*	**40 mg/kg bw** (exp. 2)*
Mean Actual dose[2]	558.8 mg/kg bw	272.7 mg/kg bw	165.9 mg/kg bw	84.7 mg/kg bw	41.6 mg/kg bw	45.1 mg/kg bw
Time [h]						
0.5	534.90	513.14	353.77	185.76	109.86	85.76
1	506.62	429.04	309.04	130.89	61.20	39.30
2	495.61	332.48	276.27	50.97	44.24	18.14
4	600.22	214.09	130.37	21.41	24.33	6.17
8	398.92	121.46	72.43	9.81	3.17	5.37
24	63.79	2.55	7.01	0.43	0.50	0.28
48	1.23	0.53	0.48	0.18	0.15	0.11
72	0.67	0.46	0.24	0.22	0.09	0.08
96	0.72	0.28	0.25	0.11	0.08	0.05
120	0.51	0.23	0.22	0.06	0.08	0.04
144	0.52	0.17	0.17	0.04	0.07	0.04
168	0.40	0.16	0.17	0.05	0.06	0.02

1Target dose is related to the acid form of BAS 351 H (Bentazone)

2 Mean Actual dose is related to the sodium salt of BAS 351 H (Bentazone) corresponding to 1.09 x dose (related to the acid form)

* The respective experiment set 1 and 2 is indicated in brackets

Calculated initial half lives were 6.15, 2.84, 2.45, 0.79, 1.23 and 0.69 hours in the 500, 250, 150, 80 and the two sets of 40 mg/kg bw dose groups, respectively. Terminal half-lives ranged between 60 and 109.13 hours for all dose groups. The calculated area under the plasma concentration time curve (AUC) ranged between 207 µg Eq*h/g to 8731 µg Eq*h/g (see Table 2.1.5-3).

Table 2.1.5-3 Pharmacokinetic parameters of ^{14}C-BAS 351 H in female Wistar rats

Target Dose[1]* [mg/kg bw]	Actual Dose[2] [mg/kg bw]	c_{max} [µg Eq/g]	T_{max} [hour]	initial half life [hour]	terminal half life [hour]	AUC [µg Eq x h/g]
500 (exp. 1)	558.8	534.90; 600.22	0.5; 4	6.15	86.62	8731
250 (exp. 2)	272.7	513.14	0.5	2.84	83.97	3156
150(exp. 1)	165.9	353.77	0.5	2.45	91.66	2144
80 (exp. 2)	84.7	185.76	0.5	0.79	60.04	502
40 (exp. 1)	41.6	109.68	0.5	1.23	109.13	303
40 (exp. 2)	45.1	85.76	0.5	0.69	60.52	207

* The respective experiment set 1 and 2 is indicated in brackets

[1] Target dose is related to the acid form of BAS 351 H (Bentazone)

[2] Mean Actual dose is related to the sodium salt of BAS 351 H (Bentazone) corresponding to 1.09 x dose (related to the acid form)

The mean blood to plasma ratios were nearly constant up to 24 hours for all dose groups with values < 1 and increased thereafter up to 168 h. Maximum mean plasma blood ratios ranged from 1.83 and 2.75 and occurred 144 or 168 h post dosing. As can be seen from AUC versus dose ratio relationships (AUC ratios of the higher dose levels compared to the AUC of the low dose experiments), the internal dose is overproportional to the oral dose (Table 2.1.5-4).

Table 2.1.5-4 AUC/dose ratios after single oral administration of ^{14}C- BAS 351 H (Bentazone) at target dose levels of 500; 250; 150, 80 and 40 mg/kg bw to female rats

Mean Actual dose[1]* [mg/kg bw]	AUC [µgEq x h/g]	AUC/dose [µgEq x h x kg bw/g x mg]
558.8	8731	15.6
272.7	3156	11.6
165.9	2144	12.9
84.7	502	5.9
45.1(exp. 2)	207	4.6
41.6 (exp. 1)	303	7.3

* The respective experiment set 1 and 2 is indicated in brackets

[1] Mean Actual dose is related to the sodium salt of BAS 351 H (Bentazone) corresponding to 1.09 x dose (related to the acid form)

To avoid overprediction of saturation, the comparison of AUC versus dose ratio relationships is performed versus the experiment with the higher AUC at the low dose level (original low dose experiment 1). Increasing the dose by a factor of 2 (from 41.6 to 84.7 mg/kg bw) results in an increase of the AUC-values by a factor of 1.7. Increasing the dose by a factor of 13.4 (from 41.6 to 558.8 mg/kg bw) results in an overproportional increase of the AUC-values by a factor of 28.8 (see Table 2.1.5-5). The observed effect

may be based on active excretion of the test substance or its metabolites with saturation at higher doses, yielding to overproportional internal doses with increasing dose when a threshold dose (saturation of excretion) is reached. Within the current study, this saturation of excretion starts between actual dose levels of 84.7 and 165.9 mg/kg bw.

Table 2.1.5-5 Dose versus AUC ratios after single oral administration of ^{14}C- BAS 351 H (Bentazone) to female rats at target dose levels of 500; 250; 150 and 80 mg/kg bw compared to a target dose level of 40 mg/kg bw (Experiment 1)

Mean Actual dose[1]* [mg/kg bw]	AUC0-∞ [µg Eq *h/g]	Dose ratio to low dose [-]	AUC ratio to AUC of low dose [-]
558.8	8731	13.4	28.8
272.7	3156	6.6	10.4
165.9	2144	4.0	7.1
84.7	502	2.0	1.7
41.6 (exp. 1)	303	1.0	1.0

* The respective experiment set 1 is indicated in brackets and taken to prevent overprediction

[1] Mean Actual dose is related to the sodium salt of BAS 351 H (Bentazone) corresponding to 1.09 x dose (related to the acid form)

Conclusion

The AUC versus dose ratio relationships indicate that the internal dose is overproportional to the oral dose. This effect may be based on active excretion of the test substance or its metabolites with saturation at higher doses, yielding to overproportional internal doses with increasing dose when a threshold dose (saturation of excretion) is reached. Within the current study, this effect starts between actual dose levels of 84.7 and 165.0 mg/kg bw (calculated as bentazone-sodium corresponding to 1.09 x dose of bentazone acid).

2.1.6 Study 6 – oral, rabbit

Anonymous 1974 (Doc. No. 74/5116)

Metabolism and balance study of [^{14}C]-BAS 351-H in rabbits; BASF Reg. Doc. No.74/5116

Testing facility: experimental work completed: 1974

Previous evaluation: in original DAR

Material and methods

Test method: This study was performed prior to implementation of specific test guidelines. Thus, its major part is not in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.

GLP: No. The study was performed prior to implementation of GLP guidelines.
Acceptance: The study is considered supplementary only since it does not comply with current standards and since there were some reporting deficiencies.
Test system: [^{14}C]-bentazone (specific activity: 4.0 µCi/mg; batch no. not available) was applied in gelatin capsules (mixed with ground feed) to three male New Zealand White rabbits (source: not known, one more male rabbit serving as control) as a single oral dose of 5 mg/kg bw. The animals had free access to food and drinking water throughout the study. They were housed in metabolism cages for feces and $C0_2$ collection. Urine was collected daily via catheters. Blood was collected prior to and at 1, 2, 3, 4.5, 5.5, 9, 11, 13.5, 20 and 24 hours after dosing. The rabbits were sacrificed 6 days after treatment.
Statistics: Statistical analysis was not performed.

Results

The total recovery of radioactivity averaged 93.5%. A total of 90.3% was excreted during the first 24 hours. Most of the radioactivity was excreted in the urine (89.7%). A small amount was excreted in the feces (3.8%). Less than 0.1% of the total radioactivity was found in the expired air. At sacrifice, radioactivity in tissues was very low (< 0.1% of dose). Tissue levels were all below 0.02 ppm.
Blood levels reached a peak 2 1/2 hours after dosing. The elimination half-life of the radioactive materials in the blood was two hours and 12 minutes. Ethyl acetate extraction of urine (0 to 24 hours) at pH 7 resulted in 5.4% of the dose in the organic phase. At pH 3, 81.1% of the dose was extractable in ethyl acetate. Thin layer chromatography of the whole urine resulted in a single spot with an R_f identical to that of bentazone and/or its 6- and 8-OH-derivatives.
Remark: Further investigation on urinary metabolites is described and evaluated in the following chapter (see section 2.1.11).

Conclusion

The results after a single oral administration of 5 mg of [^{14}C]-bentazone/kg bw to male rabbits were substantially similar to those obtained with rats. About 90% of the radioactivity administered was found in the urine and nearly 4% in the feces within 24 hours. 6 days after dosing, tissue residues were considerably low.

2.1.7 Study 7 – dermal, rat

Anonymous 1985 (Doc. No. 85/299)

Dermal absorption of [^{14}C]-bentazone in rats; BASF Reg. Doc. No. 85/299
Testing facility: experimental work completed: 08/1985
Previous evaluation: in original DAR

Material and methods

Test method: No specific test guideline was mentioned but the major part of the study is in compliance with the demands of Directive 87/304/EEC, part B, May 30, 1988.

Deviations: Metabolism was not examined. However, this is usually done after oral but not after dermal application.

GLP: When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP.

Acceptance: The study is considered acceptable.

Test system: [^{14}C]-bentazone (radiochemical purity > 97%; specific activity: 10.81 mCi/mM; batch no. not submitted) and non-radiolabelled bentazone (batch no. CH 3 8102 0) were converted to the sodium salt and applied as aqueous solution to the shaved back of male adult Sprague-Dawley (CD) rats (source: Charles River, Margate, England) for different periods ranging from 0.5 up to 10 hours under occlusive dressing. The dose levels were adjusted to 0.002; 0.02; 0.2 and 2 mg free acid/cm^2 corresponding to dermally applied doses of 0.12; 1.2; 12 and 12 0 mg/kg bw. The animals received standardized diet and water ad libitum. At each dose level, groups of 4 animals were sacrificed at 0.5, 1, 2, 4 and 10 hours after dosing. The remaining 4 rats per group were transferred to metabolism cages after coverings had been removed and back was washed with methanol at 10 hours after dose application. From these animals, samples of urine and feces were collected during 10-24, 24-48 and 48-72 hour intervals. At 72 hours after dosing, rats were killed and samples of blood and tissues were taken for analysis.

In addition, concentrations of radioactivity in liver and plasma and excretion of radioactivity in urine and feces after oral administration of bentazone sodium salt at a dose level equivalent to 4 mg free acid/kg bw to male rats have also been determined. The orally dosed animals were allocated to groups of 4 per dose level and sacrificed at the same times after dosing as indicated for the dermal application groups. Rats scheduled for termination after 72 hours were housed in metabolism cages. Urine and feces were collected separately during 0-6, 6-12, 12-24, 24-48 and 48-72 hours.

Statistics: Statistical analysis was not performed.

Results

Only a small amount of the test compound was absorbed following dermal application (see Table 2.1.7-1). This absorbed part was rapidly eliminated with urinary excretion being the main route. Less than 0.1% of the dose was excreted via the feces within 10 hours. After a 4 mg/kg bw oral dose of [^{14}C]-bentazone sodium salt, a mean of 90% of the total dose was excreted in the urine during 72 hours. A major part (85%) was excreted during the first 12 hours already. These results clearly indicated that the oral dose was extensively and rapidly absorbed and eliminated. The time course of radioactive plasma levels at the different times is summarised in Table 2.1.7-2 confirming the limited extent of percutaneous absorption.

Table 2.1.7-1: Mean total absorption and urinary excretion (given in parentheses for the 72 hour sacrifice time only) of bentazone following dermal and oral application (expressed as % of the total dose)

Sacrifice time (hours)	0.5	1	2	4	10	72
0.12 mg/kg bw, dermal	0.14	0.07	0.18	0.52	1.20	1.23 (1.16)
1.2 mg/kg bw, dermal	0.45	0.39	0.32	0.49	0.76	1.90 (1.77)
12 mg/kg bw, dermal	0.03	0.14	1.-37	1.11	1.54	1.46 (1.38)
120 mg/kg bw, dermal	0.68	0.61	0.51	0.62	0.74	0.79 (0.77)
4 mg/kg bw, oral	-	-	-	-	-	92.20* (90.35)

* total absorption calculated for this group only

Following dermal application, the proportion of the dose remaining in skin and fur was much greater, in particular at the lower dose levels. After washing with cotton wool swambs moistened with water, means of 61; 28; 13 and 2%, respectively, remained on the treated skin of animals sacrificed at 72 hours. This finding might suggest that a certain amount of test compound could not be easily removed from the skin or fur.

Table 2.1.7-2: Mean plasma concentrations of bentazone following dermal and oral application (expressed as µg/ml)

Sacrifice time (hours)	0.5	1	2	4	10	72
0.12 mg/kg bw, dermal	0.0006	0.0008	0.0006	0.0005	<0.0005	<0.0005
1.2 mg/kg bw, dermal	0.0227	0.0203	0.0079	0.0038	0.0015	<0.0011
12 mg/kg bw, dermal	0.059	0.179	0.196	0.026	0.021	<0.011
120 mg/kg bw, dermal	1.86	3.28	0.77	0.35	0.18	<0.10
4 mg/kg bw, oral	7.07	6.29	3.46	1.84	0.166	0.0041

Mean concentrations of radioactivity in tissues after dermal application of 0.12; 1.2 and 12 mg/kg bw were generally low and mostly below the level of exact determination. Mean concentrations of radioactivity in tissues after dermal application of 120 mg/kg bw at the different times of sacrifice are tabulated below (Table 2.1.7-3). The concentrations in the eye, brain and testes after dermal application were considerably low and frequently below the level of exact determination.

Table 2.1.7-3: Tissue residues after dermal application of 120 mg bentazone/kg bw (expressed as µg free acid equivalents/g tissue)

Sacrifice time (hours)	**0.5**	**1**	**2**	**4**	**10**	**72**
Liver	0.68	1.11	0.36	0.27	0.27	< 0.23
Kidney	2.43	3.98	1.82	0.46	0.29	< 0.23
Gastro-Intestinal tract	0.31	0.41	0.28	0.33	0.32	< 0.25

After oral administration of the 4 mg/kg bw dose, mean concentrations of radioactivity in the liver were 2.46; 1.94; 1.25; 0.789; 0.117 and < 0.009 µg/g, respectively. Other tissues were not analysed for residue levels in this group.

Conclusion

Only about 1% - 2% of the amount applied dermally was absorbed. In contradiction, 90% of the test substance administered orally was excreted with the urine within 72 hours confirming the high degree of absorption following oral administration. 72 hours after dermal application even of the highest dose, only traces of radioactivity were present in the animal body. Elimination of the dermally absorbed amount was rapid and effective, too, with the urine being the main route.

2.1.8 Study 8 – oral, rat

Anonymous 1987 (Doc. No. 87/0429)

The biokinetics and metabolism of [^{14}C]-bentazone in rats; BASF Reg. Doc. No.87/0429

Testing facility: date of experimental work completed: 6 August 1987

Remark: The pharmacokinetic part of this study is described and evaluated under section 2.1.1.

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed according EPA/FIFRA, Subdivision F,85-1. Thus, the study is in compliance with the demands of Directive 87/302/EEC,part B, May 30, 1988.

GLP: When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP.

Acceptance: The study is considered acceptable.

Objective: see Section 2.1.1.

Test system:

Test materials: 1.[U-phenyl-^{14}C]-bentazone, radiochemical purity > 97%; suspension in 1.5% CMC; 2.[U-phenyl-^{14}C]-bentazone sodium salt, radiochemical purity > 97% (batch no. not available); solution in water (for oral doses) or in 0.9% NaCl-solution (for intravenous application).

Metabolism was studied in male and female CD rats (source: Charles River, Margate, Kent, England). Metabolite characterization studies were performed with urine samples collected during the first 24 hours post application of single or the last of repeated doses of bentazone (for study design, description of the various study groups and further details see section 2.1.1). Metabolites in bile and feces were not characterized. Characterization of metabolites in tissues was done in liver and kidney of rats sacrificed six hours after the last of seven daily oral doses of [phenyl-U-^{14}C]-bentazone (dose group 12 in Table 2.1.1-1). Structural characterization of parent bentazone, the major urinary radioactive component, was done by means of GC/MS and by high performance liquid chromatography (HPLC) followed by MS. The MS spectra were found to correspond to that of a synthetic standard of bentazone. Quantification of metabolites in raw urine of the various dose groups of the main study was carried out by HPLC; fractions were identified by co-chromatography with standard bentazone, 6-OH-bentazone and 8-OH-bentazone. Additionally, representative urine samples were treated with β-glucuronidase/sulfatase to assess the extent of conjugation of the bentazone metabolites.

Results

The result of HPLC analysis of rat urine samples collected over a 24-hour period are presented in Table 2.1.8-1. Unchanged bentazone, as the major proportion, and small amounts of 6- and 8-OH-bentazone were identified in the urine. There were no major dose-dependent or pre-treatment-dependent differences among groups. The level of glucuronide/sulfate conjugation was either negligible or non-existent. The only metabolite detected in tissue samples was parent bentazone. The hydroxylated metabolites of bentazone were not seen in the tissue extracts presumably because the method was not sensitive enough for detection of metabolites accounting for < 10% of the dose.

Table 2.1.8-1 Quantification of metabolites in urine of rats dosed with [U-phenyl-^{14}C]-bentazone

Recovery as percent of dose								
	i.v. 4.1 mg/kg bw		**oral 3.8 mg/kg bw**		**oral 205 mg/kg bw**		**oral pre-treatment plus**	
Metabolite	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
6-OH-bentazone	0.98	-	5.91	6.34	5.44	2.43	2.14	0.13
8-OH-bentazone	0.23	-	0.16	-	-	-	-	-
bentazone (parent)	90.22	88.95	80.63	77.37	86.53	88.28	91.02	85.06
Other*	-	-	-	-	-	-	0.13	-
Total	91.43	88.95	86.70	83.71	91.97	90.71	93.29	85.19
Unaccounted* *	8.57	11.05	13.3	16.29	8.03	9.29	6.71	14.81
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* Unspecified.

** Includes unidentified material in feces, bile, tissues plus radioactive losses.

Conclusion

An investigation of urinary metabolites after the different dosing regimens studied showed a consistent pattern of bentazone being excreted in urine mostly unchanged (77% to 91% of total dose). The identity of this major excretion product was confirmed by co-chromatography with authentic bentazone and by mass spectrometry of the isolated radiolabelled compound. The minor urinary metabolite was shown to co-chromatograph with authentic 6-OH-bentazone. This component accounted for 1% to 6% of the dose and was clearly distinguished from the 8-OH-isomere by the chromatographic system used. The latter one was found only in male rats in traces (concentrations ranging from 0.16% to 0.23% of total dose). In tissues, only parent bentazone was detected, however, this could be due to methodical limitations, too.

2.1.9 Study 9 – oral rat

Anonymous 1986 (Doc. No. 86/090)

Report on the investigation of urinary metabolites of bentazone in the rat; BASF Reg. Doc. No. 86/090

Testing facility: experimental work completed: 1986

Previous evaluation: in original DAR

Material and methods

Test method: No specific test guideline was mentioned but the major part of the study is in compliance with the demands of Directive 87/304/EEC, part B, May 30, 1988

Deviations: Only urinary metabolites were examined. Metabolic pathway or parameters of pharmacokinetics were not investigated in this study.

GLP: When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP.

Acceptance: The study is considered acceptable.

Test system: A single oral dose of [U-phenyl-^{14}C]-bentazone sodium salt (batch no not available, radiochemical purity > 99%) was administered as aqueous solution to adult male CD rats (source: Charles River, Margate, UK) at a dose level equivalent to 4 mg free acid/kg bw. Rats were sacrificed at different times (4 per group) up to 72 hours after dosing. Radioactivity in the urine was checked during 0-6, 6-12 and 12-24 hour intervals post administration. Metabolite pattern was investigated by TLC in combined urine samples collected during these intervals.

Statistics: Statistical analysis was not performed.

Results

After oral doses of [^{14}C]-bentazone sodium salt at a dose level of 4 mg/kg bw, most of the radioactivity in urine corresponded to unchanged bentazone (free acid). Means of 65%, 15% and 3% of the total dose were excreted in the urine as unchanged bentazone during 0 hour - 6 hours, 6 hours - 12 hours and 12 hours - 24

hours, respectively, in sum (83%) representing more than 90% of all radioactivity excreted in the urine. A mean of about 2% of total dose corresponded to 6-OH-bentazone. Most of the remaining radioactivity (ca. 2% dose) was associated with polar material at the origin of the chromatogram. The reference compound 8-OH-bentazone did not correspond to any of the radioactive components found in this experiment. These results indicate that bentazone administered by the oral route is excreted in the urine almost entirely unchanged (83% of total dose), with traces (2%) of 6-OH-bentazone and polar material.

Conclusion

After oral administration of 4 mg/kg bw of [^{14}C]-bentazone sodium salt to 24 CD rats, the 24-hour urine was found to have 2% of 6-hydroxy-bentazone in addition to approximately 83% of unchanged bentazone.

2.1.10 Study 10 – oral, rat

Characteristics

Reference	:	Anonymous 2011c (Doc No 2010/1027274) Anonymous 2011d (Doc No 2011/1197846)	exposure	:	Single oral
type of study	:	Metabolism study in rat	doses	:	Single dose 200 mg/kg bw
year of execution	:	2009-2011	vehicle	:	0.5% carboxymethylcellulose and 1% cremophor
test substances	:	^{14}C-Bentazone (batch 210-2301), specific activity 5.15 MBq/mg, purity 96.5%; unlabelled bentazone (batch 01893-210), purity 99.8%	GLP statement	:	yes
Route	:	oral	guideline	:	According to OECD 417
Species	:	Rat, Wistar	acceptability	:	yes
group size	:	4/sex	Previous evaluation		Submitted for renewal (supplementary)

Study design

Four male and four female rats were dosed once orally with a mixture of ^{14}C-labelled and unlabelled bentazone at a nominal dose level of 200 mg/kg bodyweight. Urine and feces were sampled at 6, 12 and 24 hours post application and thereafter in 24 hour intervals, until 168 hours. Samples were pooled for males and females separately. No feces were collected from female rats at 6 hours, as there was no defecation in the respective time period. The animals were sacrificed in a carbon dioxide chamber seven days after dosing and the metabolism cages were subsequently washed. All the samples (urine, feces, carcasses and cage wash) were frozen and stored at -18°C prior to analysis. For the quantitation of radioactivity in liquid samples, a liquid scintillation counter was used. In case of solid samples, aliquots of the homogenised samples were weighed into combustion cones and combusted by means of an automatic sample oxidiser. The $^{14}CO_2$ formed during combustion was trapped by an absorption liquid and the collected radioactivity was measured by LSC. For the determination of the metabolic patterns, metabolite identification and quantitation of metabolites, HPLC methods were applied.

Results

Excretion and distribution of radioactivity

A summary of the excretion balance is shown in Table 2.1.10-1. Excretion was nearly complete within 72 hours. In both male and female rats, the vast majority of radioactivity was excreted via urine (66.08% and 65.01% of the dose, respectively). The excretion was fast and nearly completed after 72 hours. A slight delay in excretion was observed in male rats, which was most likely due to the low urination rate of male rats during the time interval 0-6 h (males: 1.9 ml, females 6.3 ml, respectively). Only a minor part of the radioactivity was excreted via feces (3.20% of the dose in male rats and 2.22% in female rats, respectively). The cage wash of male and female rats contained 11.31% and 9.14% of the dose, respectively. Only minor amounts of radioactivity were found in organs and animal remains accounting for 0.41% and 0.40% of the dose (male and female rats, respectively).

Table 2.1.10-1 Excretion balance after oral administration of ^{14}C-bentazone

	Males	**Females**
	[% of the dose]	**[% of the dose]**
Urine 0-6 h[1)]	7.52	25.06
Urine 6-12 h[1)]	20.10	5.51
Urine 12-24 h	16.59	13.24
Urine 24-48 h	8.21	12.79
Urine 48-72 h	9.61	3.82
Urine 72-96 h	1.86	1.50
Urine 96-120 h	1.15	1.16
Urine 120-144 h	0.60	1.03
Urine 144-168 h	0.45	0.90
Total urine 0-168 h	**66.08**	**65.01**
Feces 0-6 h	0.01	-[2)]
Feces 6-12 h	0.02	0.07
Feces 12-24 h	1.22	0.83
Feces 12-48 h	0.63	0.83
Feces 48-72 h	0.71	0.24
Feces 72-96 h	0.20	0.05
Feces 96-120 h	0.09	0.05
Feces 120-144 h	0.12	0.10
Feces 144-168 h	0.20	0.06
Total feces 0-168 h	**3.20**	**2.22**
Total excreted	**69.28**	**67.23**
Liver	0.003	0.002
Kidney	0.002	0.001

	Males	**Females**
	[% of the dose]	**[% of the dose]**
Animal remains	0.401	0.398
Cage wash	11.311	9.142
Total	**81.00**	**76.77**

1) The slightly delayed excretion via urine in male rats was probably due to the low urination rate of the male rats during time interval 0-6 h (males: 1.9 mL urine vs. females: 6.3 mL)

2) No defecation in the respective time period

Metabolites in urine

The composition of radioactivity in urine samples of male rats of individual time intervals is shown in Table 2.1.10-2. For all time intervals the unchanged parent compound represented the main component (from 0.45% to 18.60% of the dose). Metabolite M351H001 was significantly less abundant than the parent compound (from not detectable to 1.49% of the dose). Up to two not identified peaks were present (each below or equal to 0.10% of the dose). Overall in male rats, 62.70% of the dose was excreted via urine as the parent compound BAS 351 H and 3.15% as metabolite M351H001. A portion of 65.84% of the dose was thus identified and an additional 0.24% of the dose was characterised by their chromatographic properties.

The composition of radioactivity in urine samples of female rats of individual time intervals is depicted in Table 2.1.10-3. For all time intervals the unchanged parent compound represented the main component (from 0.90% to 24.63% of the dose). Metabolite M351H001 was significantly less abundant than the parent compound (from not detectable to 0.29% of the dose). Up to three not identified peaks were present (each below or equal to 0.14% of the dose). In female rats, 63.97% of the dose was thus excreted via urine as the parent compound BAS 351 H, what was comparable to male rats. However, the amount of metabolite M351H001 was somewhat below the amount of the same metabolite in urine of males (male: 3.15% versus female: 0.52% of the dose). Overall in female rats, a portion of 64.49% of the dose was identified and an additional 0.51% of the dose was characterised by their chromatographic properties.

Table 2.1.10-2 Composition of radioactivity in urine of male rats after oral administration of ^{14}C-bentazone

	Composition of Radioactivity in % of Applied Dose									
Time Interval [h]	0-6	6-12	12-24	24-48	48-72	72-96	96-120	120-144	144-168	Sum
Identified										
BAS 351 H	7.15	18.60	15.93	7.79	9.21	1.81	1.15	0.60	0.45	62.70
M351H001	0.37	1.49	0.66	0.33	0.26	0.03	n.d.	n.d.	n.d.	3.15
Total identified	7.52	20.10	16.59	8.13	9.47	1.84	1.15	0.60	0.45	65.84
Characterised by HPLC										
Up to two peaks (each below or equal to 0.10% dose)	n.d.	n.d.	n.d.	0.08	0.14	0.01	n.d.	n.d.	n.d.	0.24

	Composition of Radioactivity in % of Applied Dose									
Time Interval [h]	0-6	6-12	12-24	24-48	48-72	72-96	96-120	120-144	144-168	Sum
Identified										
Total identified and characterised	7.52	20.10	16.59	8.21	9.61	1.86	1.15	0.60	0.45	66.08

Table 2.1.10-3 Composition of radioactivity in urine of female rats after oral administration of ^{14}C-bentazone

	Composition of Radioactivity in % of Applied Dose									
Time Interval [h]	0-6	6-12	12-24	24-48	48-72	72-96	96-120	120-144	144-168	Sum
Identified										
BAS 351 H	24.63	5.40	13.24	12.40	3.71	1.50	1.16	1.03	0.90	63.97
M351H001	0.29	0.03	n.d.	0.17	0.03	n.d.	n.d.	n.d.	n.d.	0.52
Total identified	24.93	5.43	13.24	12.57	3.73	1.50	1.16	1.03	0.90	64.49
Characterised by HPLC										
Up to three peaks (each below or equal to 0.14% dose)	0.13	0.08	n.d.	0.22	0.08	n.d.	n.d.	n.d.	n.d.	0.51
Total identified and characterised	25.06	5.51	13.24	12.79	3.82	1.50	1.16	1.03	0.90	65.01

Metabolites in feces

The composition of radioactivity in feces methanol extracts of male rats of the three analysed time intervals is depicted in Table 2.1.10-4. For all analysed time intervals the unchanged parent compound represented the main component (0.52%, 0.17% and 0.18% of the dose). Metabolite M351H001 (6-OH-bentazone) was less abundant than the parent compound for all time intervals (0.20%, 0.14% and 0.08% of the dose). Metabolite M351H002 (8-OH-bentazone) was considerably less abundant than the parent compound (from not detectable to 0.06% of the dose). Up to four not identified peaks were present (each below or equal to 0.13% of the dose). The composition of radioactivity in feces water extracts of males of the three analysed time intervals is depicted in Table 2.1.10-4. Analysis of all three time intervals resulted in series of peaks of different polarity, which were not identified but characterised by their chromatographic properties (up to 39 peaks, each below or equal to 0.03% of the dose). Overall, 0.86% of the dose was excreted via feces as the parent compound BAS 351 H, 0.42% as metabolite M351H001 and 0.10% as metabolite M351H002. Portions of 0.29% of the dose (methanol extract) and 0.24% (water extract) were characterised by HPLC.

The composition of radioactivity in feces methanol extracts of female rats of the two analysed time intervals is depicted in Table 2.1.10-5. For all analysed time intervals the unchanged parent compound represented the main component (0.43% and 0.25% of the dose). Metabolite M351H001 was significantly less abundant than the parent compound (0.05% of the dose, detected only for time interval 12-24 h). The amount of metabolite M351H001 was somewhat below the amount of the same metabolite in feces methanol extract of males

(male: 0.42% versus female: 0.05% of the dose). Metabolite M351H002 was also significantly less abundant than the parent compound (0.05% of the dose, detected only for time interval 12-24 h). Up to three not identified peaks were present (each below or equal to 0.08% of the dose). The composition of radioactivity in feces water extracts of female rats of the two analysed time intervals is depicted in Table 2.1.10-5. Analysis of the two time intervals resulted in series of peaks of different polarity, which were not identified but characterised by their chromatographic properties (up to 36 peaks, each below or equal to 0.03% of the dose). Overall, 0.68% of the dose was excreted via feces as the parent compound BAS 351 H, 0.05% as metabolite M351H001 and 0.05% as metabolite M351H002. Portions of 0.22% of the dose (methanol extract) and 0.23% (water extract) were characterised by HPLC.

Table 2.1.10-4 Composition of radioactivity in feces of male rats after oral administration of ^{14}C-bentazone

	Composition of Radioactivity in % of Applied Dose			
Time Interval [h]	**12-24**	**24-48**	**48-72**	**Sum**
Identified	**Feces Methanol Extract**			
BAS 351 H	0.52	0.17	0.18	0.86
M351H001	0.20	0.14	0.08	0.42
M351H002	0.06	0.03	n.d.	0.10
Total identified	**0.78**	**0.34**	**0.26**	**1.38**
Characterised by HPLC				
Up to four peaks (each below or equal to 0.13% dose)	0.13	0.05	0.11	0.29
Total identified and characterised	**0.91**	**0.39**	**0.37**	**1.67**
Characterised by HPLC	**Feces Water Extract**			
Up to 39 peaks (each below or equal to 0.03% dose)	0.10	0.07	0.07	0.24

Table 2.1.10-5 Composition of radioactivity in feces of female rats after oral administration of ^{14}C-bentazone

	Composition of Radioactivity in % of Applied Dose		
Time Interval [h]	**12-24**	**24-48**	**Sum**
Identified	**Feces Methanol Extract**		
BAS 351 H	0.43	0.25	0.68
M351H001	0.05	n.d.	0.05
M351H002	0.05	n.d.	0.05
Total identified	**0.53**	**0.25**	**0.78**

	Composition of Radioactivity in % of Applied Dose		
Time Interval [h]	**12-24**	**24-48**	**Sum**
Identified	**Feces Methanol Extract**		
Characterised by HPLC			
Up to three peaks (each below or equal to 0.08% dose)	0.03	0.19	0.22
Total identified and characterised	**0.56**	**0.44**	**1.01**
Characterised by HPLC	**Feces Water Extract**		
Up to 36 peaks (each below or equal to 0.03% dose)	0.11	0.12	0.23

Metabolic Pathway

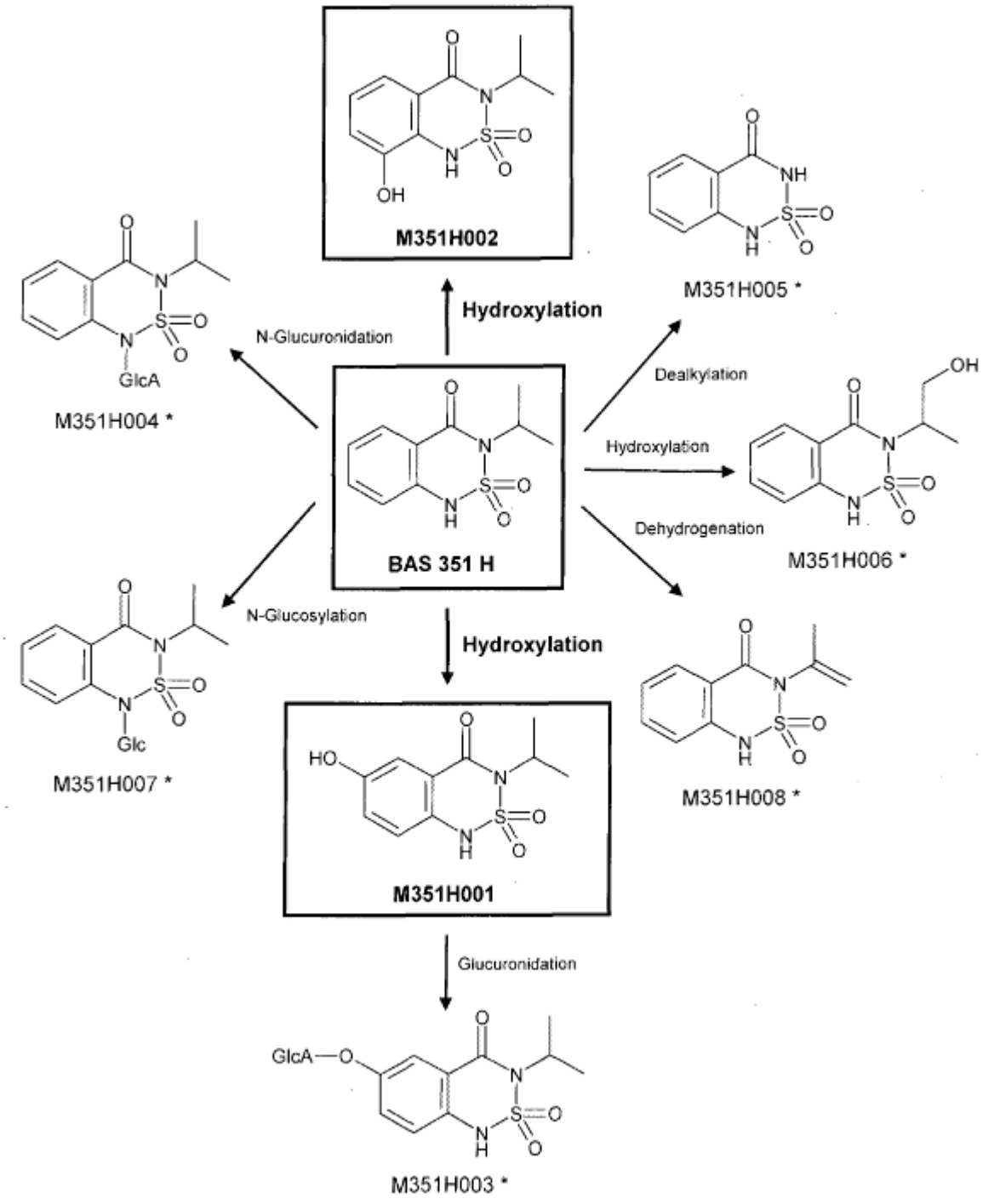
The overall metabolic pathway is depicted in Figure 2.1.10-1. All metabolites identified in the course of this study are shown. The major transformation steps in the pathway are hydroxylation of the benzothiadiazine ring at position six and eight.

The major part of BAS 351 H was excreted via urine as the unchanged parent compound. The two metabolites present in sufficient amounts for quantification resulted from hydroxylation of BAS 351 H at position six (M351H001) and position eight (M351H002). Further metabolites were present only in trace amounts: M351H003 resulted from glucuronidation of the hydroxyl group of metabolite M351H001 and M351H004 from glucuronidation of the nitrogen at position one. Dealkylation of the parent compound BAS 351 H led to metabolite M351H005. Metabolite M351H006 was formed by hydroxylation of one primary carbon atom of the parent compound. N-glucosylation of BAS 351 H resulted in metabolite M351H007 and dehydrogenation of the alkyl moiety of the parent compound in metabolite M351H008.

Conclusion

Bentazone (BAS 351 H) was metabolised to a rather small extent after a single oral high dose (200 mg/kg bw) application and was primarily excreted via urine as the unchanged parent compound. Excretion and metabolism was very similar in both sexes and 63.56% and 64.65% of the dose were excreted as unchanged BAS 351 H (bentazone) in male and female rats, respectively. Hydroxylation of the parent compound resulted in small amounts of metabolite M351H001 (6-OH-bentazone), which was excreted at levels of 3.57% and 0.58% of the dose in males and females. Metabolite M351H002 (8-OH-bentazone) was present in male and female rats at even lower levels as its isomer M351H001 (0.10% and 0.05% of the dose). The remaining six metabolites (M351H003, M351H004, M351H005, M351H006, M351H007 and M351H008) were present only in trace amounts.

Figure 2.1.10-1 Proposed metabolic pathway



* Metabolites were identified by HPLC-MS. Identification and quantitation with HPLC was not feasible due to negligible amounts.

2.1.11 Study 11 – oral, rabbit

Anonymous 1974 (Doc. No. 74/9000)

Investigations of rabbit urine and feces after oral administration of [^{14}C] bentazone; BASF Reg. Doc. No. 74/9000

Testing facility: experimental work completed: 1974

Previous evaluation: in original DAR

Material and methods

Test method: This study was performed prior to implementation of specific test guidelines. Thus, the major part of the study is not in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.

GLP: No. The study was performed prior to implementation of GLP guidelines

Acceptance: The study is considered supplementary only since it does not comply with current standards.

Test method: Urine from one male NZW rabbit treated in an abovementioned study (Roger and Davis, 1974; see 2.1.6) was collected for 72 hours following an oral dose of [^{14}C]-bentazone (5 mg/kg bw, specific activity: 0.94 mCi/mmole = 4.0 µCi/mg, batch no. not available) and examined for the test compound and its possible metabolites.

Statistics: Statistical analysis was not performed.

Results

Most (76.7%) of the administered radioactivity was found in the 0 hour to 24 hours urine sample. More than 99% of the radioactivity totally recovered in the urine was in form of the unchanged parent material. Less than 1% of the radioactivity recovered could be assigned to the two metabolites 6- and 8-OH-bentazone. The hydroxy compounds were clearly eliminated in their free form in the urine and not as conjugates with glucuronic acid.

Conclusion

It was clearly confirmed that most of the applied radioactivity was excreted in the urine within 24 hours. Bentazone was eliminated primarily in unchanged form. In addition, traces of two metabolites (6- and 8-OH-bentazone) were detected.

2.1.12 Study 12 – intravenous, mouse

Anonymous 1974 (Doc. No. 74/5117)

Metabolism of bentazone in the mouse (mus musculus); BASF Reg. Doc. No. 74/5117

Testing facility:; experimental work completed: 1974

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed prior to implementation of specific test guidelines. Thus, the major part of the study is not in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.
GLP: No. The study was performed prior to implementation of GLP guidelines
Acceptance: The study is scientifically valid. However, with respect to the year when the study was performed and to reporting deficiencies, it is considered supplementary only.
Test system: [^{14}C]-bentazone (batch no. not available; specific activity 3.09 µCi/µMol); was administered intravenously as solution in corn oil to C57 mice (source: Jackson Laboratories, UK) at a dose level of 2 00 ul per animal. The number of animals and the time of termination were not submitted. Mice were housed in metabolism cages and provided with feed and water ad libitum. Urine and feces were collected approximately every 8 hours.
Statistics: Statistical analysis was not performed.

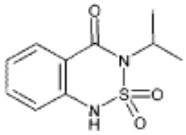
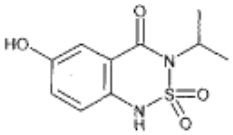
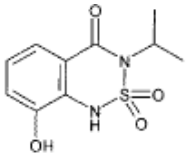
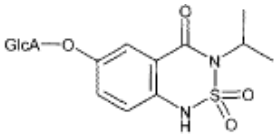
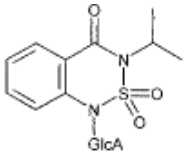
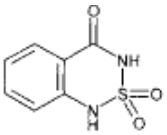
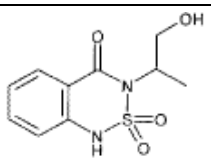
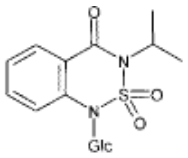
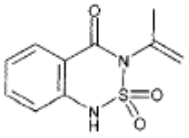
Results

The total radioactivity was excreted rapidly in the urine, with 92% being excreted within 24 hours and 95% being excreted within 48 hours. Only traces (<1%) of radioactivity were found in the feces. Analysis of the urinary metabolitesby thin layer chromatography showed that unchanged bentazone was the major constituent of the urine at all sampling intervals. A total of 76.83 + 3.61% of the radioactivity recovered in the first seven hours was identified as bentazone. At 24 hours, bentazone decreased to 49.73 + 9.92% with a corresponding increase in all metabolites. At 48 hours after dosing, the amount of unchanged parent compound had increased again up to approximately 56%. The titer of an unknown compound closely followed the rise and fall of bentazone during the study.

Conclusion

More than 95% of the radioactivity administered was recovered in the urine within 48 hours. Only traces were excreted via the feces. Unchanged bentazone was the major constituent in urine. However, investigations on metabolite pattern might suggest that bentazone in mice following intravenous application is metabolised to a greater extent than in rats or rabbits.

2.1.13 List of identified metabolites

Metabolite Designation (Code)	Molecular Mass	Structure	Urine	Faeces
BAS 351 H (M351H000)	M = 240		X	X
M351H001	M = 256		X	X
M351H002	M = 256		X	X
M351H003	M = 432		X	-
M351H004	M = 416		X	-
M351H005	M = 197		X	-
M351H006	M = 256		-	X
M351H007	M = 402		X	-
M351H008	M = 238		X	-

3. HEALTH HAZARDS

3.1 Acute toxicity – oral route

3.1.1 Animal data

3.1.1.1 Study 1 - Rat

Anonymous 1983 (Doc. No. 83/114)

Bentazone: Rat/oral - report on the study of acute oral toxicity; BASF Reg. Doc. No. 83/114

Testing facility: experimental work completed 06/1982

Previous evaluation: in original DAR

Material and methods

Test method: The test procedure to a great extent followed OECD guideline 401 and there were no deviations to Directive 92/69/EEC, part B, December 29, 1992.

GLP: No. When the study was performed, GLP was not compulsory.

Acceptance: The study is considered acceptable.

Test system: Bentazone (purity 93.9%, batch no. not available) was administered by gavage as 0.5% aqueous CMC solution to non-fed male and female Wistar rats (source: Dr. Thomae GmbH, Biberach) at dose levels of 825; 1210; 1780 and 2610 mg/kg bw. Dose groups consisted of five animals per sex. Following treatment, rats were observed for 15 days and body weight was determined after 4, 6 and 13 days .

Statistics: Probit analysis.

Results

Clinical findings: Mortality rates (male/female animals) were as follows: 5/5 (2610 mg/kg bw), 2/4 (1780 mg/kg bw), 0/1 (1210 mg/kg bw). All other animals survived the 15-day observation period. The LD_{50} values were calculated to be: LD_{50} , males: about 1780 mg/kg bw LD_{50}, females: 1470 (1080 - 1990 C.I.) mg/kg bw LD_{50}, males and females combined: 1640 (1400 - 1920 C.I.) mg/kg bw. Signs of toxicity noted in the 825 mg/kg bw to 2610 mg/kg bw dose groups comprised dyspnoea, apathy, staggering gait, opisthotonus, cachexia and poor general state. However, body weight development was not compromised.

Gross pathology: Necropsy findings in animals that died were general congestion, spot like hyperemia in lungs, loam-colored liver and very slight indications of acinar pattern in this organ, bloody ulcerations in the stomach, contents of intestines mixed with blood and gray kidneys. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

Conclusions

Under the conditions of this study the acute oral LD_{50} was found to be 1640 mg/kg bw for male and female animals combined. Therefore, the substance was assessed as **moderately toxic.**

3.1.1.2 Study 2 - Rat

Anonymous 1983 (Doc. No. 83/113)

Report on the study of acute oral toxicity in the rat of Reg. No. 51 929; BASF Reg. Doc. No. 83/113

Testing facility: date of experimental work completed: 21 June 1983

Previous evaluation: in original DAR

Material and methods

Test method: The test procedure to a great extent complied with OECD guideline 401 and there were no deviations from Directive 92/69/EEC, part B, December 29, 1992.

GLP: No. When the study was performed, GLP was not compulsory.

Acceptance: The study is considered acceptable.

Test system: Bentazone technical (batch no. not available) was administered as 0.5% aqueous CMC solution by oral gavage to non-fed male and female Wistar rats (source: Dr. Thomae GmbH, Biberach). Dose levels were 562; 825; 1210; 1780 and 2610 mg/kg bw. Five animals per sex and dose group were tested. Treatment was followed by a 15 day observation period. Body weight determination took place after 3, 4, 6, 7 and 13 days.

Statistics: Probit analysis.

Results

Clinical findings: Mortality rates (male/female animals) were as follows: 5/3 (2610 mg/kg bw), 2/3 (1780 mg/kg bw), 1/1 (1210 mg/kg bw), 0/2 (825 mg/kg bw) . All other animals survived to the end of the observation period. Calculation of LD_{50} revealed the following values:

LD_{50}, males: 1780 mg/kg bw;

LD_{50}, females: 1790 mg/kg bw;

LD_{50}, males and females combined: 1710 mg/kg bw.

Signs of toxicity noted in the 825 mg/kg bw to 2610 mg/kg bw dose groups comprised dyspnoea, apathy, cachexia, piloerection, staggering and poor general state. Females of the 1780 mg/kg bw dose group and all animals of the 562 mg/kg bw dose group did not show any symptoms. In the survivors, the expected body weight gain has been observed in the course of the study.

Gross pathology: Necropsy findings in animals that died intercurrently were general congestion, spot like hyperemia and slight emphysema in lungs, ulcers and hemorrhages in the gastro-intestinal tract, anemic color

and slight acinar pattern of the liver. In one high-dose male, kidneys were sand-colored and adrenals loam-colored additionally.

No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

Conclusion

Under the conditions of this study the acute oral LD_{50} was found to be 1710 (1340 - 2460 C.I.) mg/kg bw for male and female animals. Thus, the material is assessed as **moderately toxic.**

3.1.1.3 Study 3 - Rat

Anonymous 1973 (Doc. No. 73/022)

Acute oral toxicity of 3-isopropyl-lH-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide to the rat; BASF Reg. Doc. No. 73/022

Testing facility: date of experimental work completed: 6 December 1973

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed prior to implementation of specific test guidelines.

Deviations from current guidelines: Individual clinical findings were not reported and body weights were not determined. No detailed description of the test method and reporting of the results.

GLP: No. The study was performed prior to implementation of GLP guidelines.

Acceptance: With respect to the abovementioned deficiencies, this study is considered supplementary only.

Test system: Male and female Sprague-Dawley rats (five per sex and group; source: Wiga Co., Sulzfeld) were administered bentazone technical (batch no. not available) as 0.8% aqueous CMC solution at dose levels of 500; 640; 800; 1000; 1250; 1600 and 2000 mg/kg bw by oral gavage. Observation period: 0-14 days. All rats were subjected to necropsy.

Statistics: according to Litchfield and Wilcoxon.

Results

Clinical findings:

Mortality rates (male/female animals) were as follows: 5/5 (2000 mg/kg bw), 4/4 (1600 mg/kg bw), 2/4 (1250 mg/kg bw) and 0/2 (1000 mg/kg bw). Most of these animals died within 24 hours post application. All other animals survived the 14-day observation period. Signs of toxicity were noted at 1000 mg/kg bw and above comprising dyspnoea and red incrustations on eyes.

Gross pathology: Necropsy findings of the animals that died intercurrently were acute congestive hyperaemia, acute cardiac dilatation (right chamber), liver putty colored with lobular pattern. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

Conclusion

Under the conditions of this study, the acute oral LD_{50} was found to be 1220 (1056 - 1409 95% C.I.) mg/kg bw for male and female animals. Therefore, the substance was assessed as **moderately toxic.**

3.1.1.4 Study 4 - Rat

Anonymous 1973 (Doc. No. 73/023)

Acute oral toxicity of the sodium salt of 3-isopropyl-lH-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide to the rat; BASF Reg. Doc. No. 73/023

Testing facility: date of experimental work completed: 6 December 1973

Previous evaluation: in original DAR

Material and methods

See the abovementioned study Anonymous 1973 (Doc. No. 73/022). However, 800 mg/kg bw was the lowest dose tested in this experiment.

Results

Clinical findings: Mortality rates (male/female animals) were as follows: 5/4 (2000 mg/kg bw), 3/2 (1600 mg/kg bw), 1/2 (1250 mg/kg bw). These animals all died within 24 hours post application. All other animals survived the 14-day observation period. Signs of toxicity noted in all dose groups were dyspnoea and prostration.

Gross pathology: Necropsy findings in animals that died intercurrently were acute congestive hyperaemia, acute cardiac dilatation (right), liver pale brown with lobular pattern, stomach slightly red and contents of stomach and intestines hematinic. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

Conclusion

Under the conditions of this study the acute oral LD_{50} was calculated to be 1480 mg bentazone sodium salt/kg bw equivalent to 1356 mg (1148 - 1601 C.I.) bentazone (free acid)/kg bw for male and female animals combined. Thus, the material is assessed as **moderately toxic.**

3.1.1.5 Study 5 - Rat

Anonymous 1978 (Doc. No. 78/053)

Acute oral, subcutaneous and intraperitoneal toxicity studies of bentazone-acid in the rat; BASF Reg. Doc. No. 78/053

Testing facility: date of experimental work completed: December 1978

Remark: Subcutaneous and intraperitoneal application trials are not evaluated in this dossier.

Previous evaluation: in original DAR

Material and methods

Test method: This study was performed prior to implementation of specific test guidelines.

Deviations from current guidelines: No individual clinical findings were submitted. Body weights were not determined. No detailed description of the test method and no detailed reporting of the results are included in the report. The LD_{50} values were calculated on day 7 mortality rate (However, no animal died between day 7 and scheduled termination).

GLP: No. The study was performed prior to implementation of GLP guidelines.

Acceptance: With respect to the abovementioned deficiencies, this study is considered supplementary only.

Test System: Bentazone (purity: 94.6%, batch no. 270 778) was orally administered as 0.5% aqueous CMC solution to ten Sprague-Dawley (CRJ:SD) rats (source: Nippon Charles River) per sex and dose group at dose levels of 1500; 1800; 2160; 2592; 3110 and 3732 mg/kg bw by gavage. Observation period: 0-14 days.

Statistics: according to Litchfield and Wilcoxon.

Results

Clinical findings:

Mortality rates (male/female animals) were as follows: 10/9 (3732 mg/kg bw), 8/7 (3110 mg/kg bw), 6/5 (2592 mg/kg bw), 6/3 (2160 mg/kg bw), 2/0 (1800 mg/kg bw). The majority of deaths occurred 30 - 60 minutes after compound administration. Signs of toxicity noted in all dose groups comprised decreased spontaneous motility, ventral position, clonic convulsions and abdominal respiration. Surviving rats started to exhibit a gradual recovery in motility 5 hours after treatment. Complete recovery required 24 hours.

Gross pathology: Necropsy did not reveal abnormalities in either intercurrently dead animals or those rats which were sacrificed at the end of the study.

Conclusion

Under the conditions of this study the acute oral LD_{50} was found to be 2340 (2208 - 2480 C.I.) mg/kg bw for male and 2470 (2058 - 2964 C.I.) mg/kg bw for female animals. Thus, in contrast to the other studies, the material was shown to exhibit a **low acute oral toxicity** in this experiment.

3.1.1.6 Study 6 - Rat

Anonymous 1969 (Doc. No. 69/0013)

Acute oral toxicity of thianon in rats; BASF Reg. Doc. No. 69/0013

Testing facility:; experimental work completed: August 1969

Previous evaluation: in original DAR

Material and methods

Test method: Study was performed prior to implementation of specific test guidelines.

GLP: No. This study was performed prior to implementation of GLP guidelines.

Acceptance: The study is considered supplementary only since individual clinical findings were not reported and body weight was not determined. In addition, no detailed description of the test method and the results are contained in the report.

Test system: Bentazone (former name thianon; batch no. not available; purity not determined) was administered as 2 - 16% aqueous tragacanth suspension by oral gavage to male and female Sprague-Dawley rats (five per sex and group; source: Gassner Co., Ottobrunn, Germany) at dose levels of 200; 400; 800; 1000; 1250 and 1600 mg/kg bw. Observation period was 7 days.

Statistics: Statistical analysis was not performed.

Results

Clinical findings: Mortality rates (male/female animals) were as follows: 5/5 (1600 mg/kg bw), 5/5 (1250 mg/kg bw), 5/3 (1000 mg/kg bw), 2/2 (800 mg/kg bw). All other animals survived the observation period. Signs of toxicity noted at 200 mg/kg bw and above comprised dyspnoea, apathy and piloerection.

Gross pathology: Necropsy findings of animals that died were anus smeared with feces and pseudomelanotic discoloration of the viscera. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

Conclusion

Under the conditions of this study the LD_{50} was found to be about 850 mg/kg bw for male and female animals. Thus, the material was assessed as **moderately toxic.** It is not clear whether the substance tested was chemically identical with currently produced bentazone. In particular, the pattern of impurities is unknown.

3.1.1.7 Study 7 - Rat

Anonymous 1972 (Doc. No. 72/051)

Bericht über die Prüfung der akuten oralen Toxizitat von Bentazon techn. an der Ratte; BASF Reg. Doc. No. 72/051

Testing facility: date of report: 1 December 1972

Previous evaluation: in original DAR

Material and methods

Test method: Study was performed prior to implementation of specific test guidelines.

GLP: No. This study was performed prior to implementation of GLP guidelines.

Acceptance: The study is considered supplementary only since individual clinical findings were not reported and body weight was not determined. In addition, no detailed description of the test method and the results are contained in the report.

Test system: Bentazone technical (batch no. not available; purity not determined) was administered as 8% aqueous CMC suspension by oral gavage to five male and female Sprague-Dawley rats (source: Ivanovas, KiSlegg, Germany)/sex and dose group. Dose levels were 400; 500; 640; 800; 1000; 1250; 1600 and 2000 mg/kg bw. After dosing, rats were observed for 14 days.

Statistics: according to Litchfield and Wilcoxon.

Results

Clinical findings: Mortality rates (male/female animals) were as follows: 5/4 (2000 mg/kg bw), 5/5 (1600 mg/kg bw), 3/3 (1250 mg/kg bw) and 4/1 (1000 mg/kg bw). Most of these animals died within 24 hours post application. All other animals survived the 14-day observation period. Signs of toxicity noted in the 1250 mg/kg bw to 2000 mg/kg bw dose groups comprised dyspnoea and apathy. The animals of the other dose groups did not show any symptoms.

Gross pathology: Necropsy findings of animals that died were cardiac dilatation and congestive hyperaemia. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

Conclusions

Under the conditions of this study the LD_{50} was found to be 1050 (847 - 1302 confidence interval) mg/kg bw for male and female animals. Thus, the material was assessed as **moderately toxic.** It is not clear whether the substance tested was chemically identical with currently produced bentazone. In particular, the pattern of impurities is unknown.

3.1.1.8 Study 8 - Rabbit

Anonymous 1969 (Doc. No. 69/005)

Acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)-2,2-dioxide (technical grade) on rabbits; BASF Reg. Doc. No. 69/005

Testing facility: date of test report: October 1969

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed prior to implementation of specific test guidelines.

GLP: No. This study was performed prior to implementation of GLP guidelines.

Acceptance: According to current standards, the study would have to be considered unacceptable. Individual clinical signs were not reported and body weight was not determined. In addition, the number of animals tested was to low and no detailed description of test method and results are contained in the report. Gross pathological examination was not performed. However, it provides aditional information regarding oral toxicity in one more animal species and is considered supplementary, therefore.

Test system: Bentazone technical (batch no. not available) was orally administered as 2.5; 5 or 20% aqueous tragacanth suspension to male and female rabbits (strain not specified; source: BASF Institute of Industrial Hygiene and Pharmacology, Ludwigshafen) at dose levels of 100; 250; 500; 1000 and 2000 mg/kg bw by gavage. One animal per sex was treated at each dose level. Recording period was 7 days following treatment.

Statistics: Statistical analysis was not performed.

Results

Clinical findings: At the upper dose levels of 2000 and 1000 mg/kg bw, both animals died. All other rabbits survived the observation period. Signs of toxicity were noted in the 100; 500 and 2000 mg/kg bw dose groups and comprised slight giddiness, anorexia and diarrhoea.

Conclusions

As compared to current standards, the study is considered unacceptable for evaluation purposes. However, **findings suggest moderate acute oral toxicity also in this species.**

3.1.1.9 Published literature: Rabbit

Moderate acute oral toxicity in rabbits is confirmed by a more recent published report (Neuschl J and Kacmar P, 1993) serving as additional information. Bentazone applied was produced in Slovakia. For male and female adult NZW rabbits, a combined LD_{50} of 1139 mg/kg bw was calculated with respiratory, cardiac and central nervous symptoms occurring (BASF Reg. Doc. #93/11411).

3.1.1.10 Study 9 - Guinea pig

Anonymous 1991 (Doc. No. 91/10147)

Report on the study of the acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinon- (4) -2 , 2-dioxide (= bentazone) in Guinea pigs; BASF Reg. Doc. No. 91/10147

Testing facility: experimental work completed: 1974 (original German report dated 7 March 1974)

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed in guinea pigs and followed a procedure that was similar to the abovementioned studies in rats. However, even for rats, no specific test guidelines did exist at that time.

GLP: No. This study was performed prior to implementation of GLP guidelines.

Acceptance: With respect to reporting deficiencies and the year when the experiment was performed, the study is considered supplementary only.

Material and methods: Bentazone technical ("free acid", batch no. not available); was administered as 4 - 16% aqueous CMC solution to male and female Guinea pigs (strain not specified; source: Baumler Co., Wolfratshausen). Groups of five animals per sex were orally dosed 400; 800; 1200; 1600 or 3200 mg/kg bw.

Statistics: Statistical analysis was not performed.

Results

Clinical finding: Mortality rates (deaths/total number of animals) were as follows: 10/10 (3200 mg/kg bw), 7/10 (1600 mg/kg bw), 6/10 (1200 mg/kg bw), 1/10 (800 mg/kg bw). All other animals survived the 14-day observation period. Most of the unscheduled deaths occured within 48 hours post application. Signs of toxicity noted in the 1200; 1600 and 3200 mg/kg bw dose groups comprised abdominal-lateral position, apathy, tachypnoea, atonia and dyspnoea. The animals of the other dose groups did not show any symptoms.

Gross pathology: Necropsy findings of animals that died intercurrently were acute congestion, acute cardiac dilatation and acute inflation of the lung. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

Conclusion

Under the conditions of this study, the LD_{50} was found to be about 1100 mg/kg bw for male and female animals. Thus, the material was assessed as **moderately toxic to guinea pigs.**

3.1.1.11 Study 10 - Guinea pig

Anonymous 1974 (Doc. No. 74/035)

Acute oral toxicity of the sodium salt of 3-isopropyl-lH-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide (bentazon) to the guinea pig; BASF Reg. Doc. No. 74/035

Testing facility: date of experimental work completed: March 7, 1974
Previous evaluation: in original DAR

Material and methods

Test method: The study was performed in guinea pigs and followed a procedure that was similar to the abovementioned studies in rats. However, even for rats, no specific test guidelines did exist at that time.
GLP: No. This study was performed prior to implementation of GLP guidelines.
Acceptance: With respect to reporting deficiencies and the year when the experiment was run, the study is considered supplementary.
Test system: Bentazone, technical, sodium salt (batch no. not available) was orally administered as 6.4 - 16 % aqueous CMC solution to male and female Guinea pigs (five per sex and group; strain not specified; source: Baumler Co., Wolfratshausen). Dose levels tested were 640; 800; 1000; 1250 and 1600 mg/kg bw.
Statistics: Statistical analysis was not performed.

Results

Clinical findings: Mortality rates (deaths/total number of animals) were as follows: 10/10 (1600 mg/kg bw), 9/10 (1250 mg/kg bw), 1/10 (1000 mg/kg bw). All these deaths occurred within the first six hours following dosing. The other animals survived the 14-day observation period. Signs of toxicity were noted in the 1250 mg/kg bw and 1600 mg/kg bw dose groups and comprised prostration, apathy and tachypnoea. The animals of the other dose groups did not show any symptoms.
Gross pathology: Necropsy findings of animals that died intercurrently were acute congestive hyperaemia, acute cardiac dilatation, emphysema in lungs with areas of hyperaemia resembling an infarct. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

Conclusion

Under the conditions of this study the LD_{50} was found to be about 1100 mg bentazone sodium salt/kg bw equivalent to about 1000 mg bentazone acid/kg bw for male and female animals. Thus, bentazone is of **moderate toxicity in guinea pigs** when applied as its sodium salt.

3.1.1.12 Study 11 - Dog

Anonymous 1970 (Doc. No. 70/017)

Acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)H-2,2-dioxide in dogs; BASF Reg. Doc. No. 70/017
Testing facility: date of test report: 8 January 1970
Previous evaluation: in original DAR

Material and methods

Test method: The study was performed in dogs and followed a procedure that was similar to the abovementioned studies in rats. However, even for rats, no specific test guidelines did exist at that time.

GLP: No. The study was performed prior to implementation of GLP guidelines.

Acceptance: With respect to the small number of animals per dose group and reporting deficiencies, this study is considered supplementary only. In addition, the LD_{50} could not be determined.

Test system: Technical grade bentazone (batch no. not available; purity not determined) was administered by oral gavage as aqueous tragacanth solution (1 - 20%) to at all 8 male and 3 female Beagle dogs (source: BASF Aktiengesellschaft, Ludwigshafen, Germany) at dose levels of 50; 100; 250; 500; 1000 and 2000 mg/kg bw. Two animals per dose were used.

Statistics: Statistical analysis was not performed.

Results

Clinical findings: Mortality rates (deaths/total number of animals) were as follows: 2/2 (2000 mg/kg bw), 1/2 (1000 mg/kg bw). The animals died within 24 hours post application. All other animals survived the 14-day observation period. Signs of toxicity noted in the 250 mg/kg bw to 2000 mg/kg bw dose groups comprised vomiting, piloerection, tremors, unsteady gait, loss of rising reflex and spasm. Animals in the lower dose groups did not exhibit any toxic symptoms.

Gross pathology: Necropsy findings in animals that died after dosing were catatonic rigor mortis, muscular cyanosis and congestion of the viscera. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

Conclusion

Due to the vomiting of the animals in the upper dose groups, it was not possible to determine the approximate acute oral LD_{50}. However, it can be assumed to exceed 500 mg/kg bw.

3.1.1.13 Study 12 - Cat

Anonymous 1970 (Doc. No. 70/016)

Acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)-2,2-dioxide to cats; BASF Reg. Doc. No. 70/016

Testing facility: date of test report: 6 March 1970

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed in cats and followed a procedure that was similar to the abovementioned studies in rats. However, even for rats, specific test guidelines did not exist at that time.
GLP: No. This study was performed prior to implementation of GLP guidelines.
Acceptance: Study is considered supplementary only due to the small number of animals in most dose groups and to reporting deficiencies. For example, sum of cats allocated to the various study groups exceeded the submitted number of all animals on study by two. By current standards, it was justified to assess the study as unacceptable. However, it provides some additional information concerning toxicity in one more mammalian species.
Test system: Bentazone technical (batch no. not available) was given as aqueous tragacanth solution (5 - 2 0%) by oral gavage to 4 male and 6 female cats (source: Institute of Industrial Hygiene and Pharmacology, Ludwigshafen, Germany) at dose levels of 250; 500; 1000 and 2000 mg/kg bw (two to six cats tested per sex and dose).
Statistics: Statistical analysis was not performed;

Results

Clinical findings: Mortalities were occurring at 500 mg/kg bw and above. Mortality rates (deaths/total number of animals) were as follows: 1/2 (2000 mg/kg bw), 1/2 (1000 mg/kg bw), 3/6 (500 mg/kg bw). The animals died between 1.5 hours and two days post application. Signs of toxicity noted in the 500 mg/kg bw to 2000 mg/kg bw dose groups comprised vomiting, transient mydriasis, staggering gait, tremor, prostration, loss of rising reflex, atony, convulsions, opisthotonus, tetanic spasm and spastic paresis. Additionally, a slight bodyweight loss was noted at all dose levels.
Gross pathology: Necropsy findings of animals that died intercurrently were foci of fatty degeneration and necrobiosis on the cut surface of the liver. No abnormalities were noted at necropsy (including histological examination of brain and spinal cord) in the animals sacrificed at the end of the study.

Conclusion

Under the conditions of this study, the **LD_{50} was found to be about 500 mg/kg bw for male and female cats.**

3.1.2 Human data

Not evaluated in this dossier.

3.1.3 Other data

Not evaluated in this dossier.

3.2 Acute toxicity – dermal

3.2.1 Animal data

Not evaluated in this dossier.

3.2.2 Human data

Not evaluated in this dossier.

3.2.3 Other data

Not evaluated in this dossier.

3.3 Acute toxicity – inhalation route

3.3.1 Animal data

Not evaluated in this dossier.

3.3.2 Human data

Not evaluated in this dossier.

3.3.3 Other data

Not evaluated in this dossier.

3.4 Skin corrosion/irritation

3.4.1 Animal data

Not evaluated in this dossier.

3.4.2 Human data

Not evaluated in this dossier.

3.4.3 Other data

Not evaluated in this dossier.

3.5 Serious eye damaging/eye irritation

3.5.1 Animal data

Not evaluated in this dossier.

3.5.2 Human data

Not evaluated in this dossier.

3.5.3 Other data

Not evaluated in this dossier.

3.6 Respiratory sensitisation

3.6.1 Animal data

Not evaluated in this dossier.

3.6.2 Human data

Not evaluated in this dossier.

3.6.3 Other data

Not evaluated in this dossier.

3.7 Skin sensitisation

3.7.1 Animal data

3.7.1.1 Study 1 - Guinea pig

Anonymous 1986 (Doc. No. 86/195)

Report on the Maximization test for the sensitizing potential of Reg. No.51 929 -bentazon in guinea pigs; BASF Reg. Doc. No. 86/195

Testing facility: experimental work completed: 02/1986

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed according to OECD 406 (Maximization test) and is in compliance with Directive 92/69EEC, part B, December 29, 1992, therefore.

Additional investigations: Test groups consisted of 20 instead of 10 animals. Three challenges instead of only one were included.

GLP: Yes. When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP.

Acceptance: The study is considered acceptable.

Test system: For induction purposes, bentazone (purity: 94.0%; batch no. MS 2 F 22) was intradermally applied as aqueous solution [5% in aqua dest. or in Freund's adjuvant/aqua dest. (1:1)] to 20 female Pirbright White Guinea pigs (source: Lippische Versuchstierzucht, Hagemann GmbH & Co. KG, Extertal, Germany). For percutaneous induction (48-hour exposure) one week later, the test substance was formulated in aqua dest. An amount of about 0.3 g of the formulation was applied per animal.
Three control groups consisting of 10 females each were intradermally induced with Freund's adjuvant/aqua dest. (1:1), pure aqua dest. or remained untreated. Percutaneous induction was not conducted, since with aqua dest. a vehicle was used that was not expected to influence the outcome of the study.
Percutaneous challenges of test and control animals with a 50% test substance preparation in aqua dest. were conducted 19 (first challenge), 26 (second challenge) and 33 days (third challenge) after intradermal induction. Only one control group was challenged all three times the others being treated twice or once only. Body weight was determined prior to intradermal induction and before study termination.
Statistics: Statistical analysis was not performed.

Results

After intradermal induction, distinct erythema and edema were observed at the injection sites of the control animals and the test animals which were applied with Freund's adjuvant/aqua dest. (1:1). The injection of test substance formulation in Freund's adjuvant/aqua dest. (1:1) or in aqua dest. also caused distinct erythema and edema in the test group. The control animals injected with aqua dest. did not show any skin reactions.
The percutaneous induction with the test substance preparation in aqua dest. caused incrustation (which resulted from the intradermal induction) in addition to distinct erythema and edema.
After the first challenge, 10/20 test animals exhibited distinct erythema. Eight of these ten animals had also slight edema. 2/20 test animals only showed slight erythema.
In the control group 1, no skin reactions were noted.
After the second challenge, 4/20 test animals showed distinct erythema and slight edema. 2/20 test animals only exhibited slight erythema. In control group 1, distinct erythema and slight edema were observed in one animal. Control group 2 showed no skin reactions.
After the third challenge, distinct erythema and slight edema were observed in 9/2 0 test animals, three of these nine animals also exhibiting superficial scurf. 6/20 animals only showed slight erythema. 1/20 animals exhibited slight erythema and edema in addition to superficial scurf.
In control group 1, 1/10 animals had distinct erythema and slight edema. Two animals only showed slight erythema. In control group 2, one animal exhibited distinct erythema and slight edema. In control group 3, no skin reactions were observed.

The results after the challenges are compiled in table 3.7.1.1-1.

Table 3.7.1.1-1: Incidences of skin findings after first, second and third challenge

	1st challenge	2nd challenge	3rd challenge
Control group 1	0/10	1/10	3/10
Control group 2	no application of test substance	0/10	1/10
Control group 3	no application of test substance		0/10
Test group	12/20	6/20	16/20

Conclusion

Under the conditions of this study and considering the described findings, **bentazone has a sensitizing effect on the skin of the guinea pig.**

3.7.1.2 Study 2 - Guinea pig

Anonymous 1986 (Doc. No. 86/221)

Report on the open epicutaneous test (OET) for the sensitizing potential of bentazone-Na 600 g/l in the Guinea pig; BASF Reg. Doc. No. 86/221

Testing facility: experimental work completed: 08/1986

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed according to OECD 406 (OET) and a method published in the literature [Klecak G, 1977, Dermatotoxicology and Pharmacology, in: Advances in Modern Toxicology, edited by Marzulli FN and Maibach HI, vol.4, 321]. It is in compliance with Directive 92/69 EEC, part B, December 29, 1992 since it is stated that other than the Maximization or Buehler tests can be performed if this is scientifically reasonable.

Deviations: OET was conducted instead of recommended Maximization or Buehler test.

GLP: Yes. When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP.

Acceptance: The study is considered acceptable.

Test system: For induction, eight female Pirbright White guinea pigs (strain: Dunkin Hartley HOE DHP K [SPF-LAC] BOE; source: Lippische Versuchstierzucht, Hagemann GmbH & Co. KG, Extertal, Germany) per group were dermally administered undiluted formulation bentazone sodium salt 600 g/l (batch no. WH 4976) or aqueous formulations (2%; 10%; 50% in aqua dest.) in the Open Epicutaneous test (OET) on intact skin. For induction, 20 applications were carried out at all. The amount applied was 0.1 ml formulation/8 cm^2. Single dermal challenge applications of the abovementioned formulations (0.025 ml/2 cm^2) were given at 28 and 42 days after first application. Two control groups were included. Body weights were determined before the study commenced and prior to sacrifice of the animals .

Statistics: Statistical analysis was not performed.

Results

Findings: During the induction phase, no skin reactions were noted in animals of the control and test groups. The number of animals with skin findings after the first challenge and after the second challenge is collated in table 3.7.1.2-1.

Table 3.7.1.2-1: Incidence and scoring of skin findings after first and second challenge

	Concentrations of								
	Induction	**1st challenge**				**2nd challenge**			
		undiluted	**50% in b**	**10% in b**	**2% in b**	**undiluted**	**50% in b**	**10% in b**	**2% in b**
Control group 1	a	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Control group 2	a	a	a	a	a	0/8	0/8	0/8	0/8
Test group 4	undiluted	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Test group 5	50% in b	2/8	2/8	0/8	0/8	4/8	3/8	0/8	0/8
Test group 6	10% in b	1/8	1/8	0/8	0/8	1/8	1/8	0/8	0/8
Test group 7	2% in b	0/8	0/8	0/8	0/8	1/8	1/8	0/8	0/8

a: no application of test substance

b: aqua dest.

x/y: number of positive reactions/total number of animals in test

The tabulated signs suggest that the application of the undiluted compound and of its 10% and 2% concentrations for induction did not cause skin reactions after challenge. Results in the test groups induced with test substance concentrations of 10% or 2% are equivocal when challenged with high concentrations. However, they do not suggest a clear sensitizing potential at these concentrations. A concentration of 50% in aqua dest. for induction and challenge is needed to cause sensitization.

Conclusion

On the basis of the described findings, a **sensitizing potential** that could be of significance under conditions in practice can be assumed **for application concentrations of 50%.** Bentazone was **not sensitizing in the OET at concentrations of 2% and 10%.**

3.8 Germ cell mutagenicity

3.8.1 In vitro date

Not evaluated in this dossier.

3.8.2 Animal data

Not evaluated in this dossier.

3.8.3 Human data

Not evaluated in this dossier.

3.8.4 Other data

Not evaluated in this dossier

3.9 Carcinogenicity

Not evaluated in this dossier. However, summaries of the carcinogencity studies are provided to support the evaluation of the endpoint reproductive toxicity.

3.9.1 Animal data

3.9.1.1 Study 1 – 2 year carcinogenicity rat

Anonymous 1985 (Doc. No. 85/433)

Studies on the 24-month chronic toxicity of bentazone in rats; BASF Reg. Doc. No. 85/433

Testing facility:; experimental work completed: 1983

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed according to OECD guideline 453 and is therefore in compliance with the demands of Directive 87/302/EEC, May 30, 1987.

Deviations: None.

GLP: Yes. When the study was performed, GLP was not compulsory. However, it is stated in the report that the study was run according to the principles of GLP.

Acceptance: The study is considered acceptable.

Test system: 50 male and female Fischer 344 Du/Crj (SPF) rats (source: Charles River Japan Co., Ltd.) per sex and dose group were administered bentazone (purity 93.9%; batch no. N 169) for two years as admixture in the diet. In addition, satellite groups comprising 10 animals per sex and dose level each were subjected to interim kill after 6 and 12 months. Dose levels were chosen on the basis of a previous range-finding study. In the main study, rats were fed doses of 0; 2 00; 800 and 4000 ppm. The animals were housed two per cage

under controlled conditions and had free access to standardized diet and tap water. The test substance/feed preparations were prepared monthly. Clinical signs and mortalities were recorded twice daily. Palpation was done on skin and abdominal organs once a week. Feed and water consumption were recorded daily. Body weight was determined weekly. Ophthalmoscopy, urinalysis, hematological and clinicochemical examinations were carried out after 6, 12 and 24 months. At the end of the study, gross-pathological and histopathological examinations were performed.

Statistics: Student's t-test for most of the values obtained. Fisher's direct computation for probability was applied to assess the incidence of clinical signs and pathological findings.

Results

Analysis: The analysis of the dietary concentrations confirmed the intended dose levels (200 ppm: 102.5% of theoretical value; 800 ppm: 100.4% of theoretical value; 4000 ppm: 102.9% of theoretical value). Substance intake: The mean substance intake is tabulated below.

Table 3.9.1.1-1: Mean substance intake during the main study

Sex	**Dose level (ppm)**	**Intake for 104 weeks (mg/kg bw)**	**Intake for 26 weeks (mg/kg bw)**	**Intake for 52 weeks (mg/kg bw)**
Males	200	9	9	12
	800	35	39	47
	4000	180	197	233
Females	200	11	12	14
	800	45	48	55
	4 000	244	249	274

General observations: Mortality was comparable in all the test groups. At the highest dose level, three male animals that died prematurely had hemorrhagic lesions. Male and female animals of the highest dose group showed a significantly lower mean body weight due to reduced body weight gain (see Table 3.9.1.1-2) and significantly increased water consumption. At 800 ppm, a transient reduction in body weight was noted for male animals and the water consumption was feguently increased in both sexes. Food consumption was significantly decreased in high dose males in some weeks. Food efficiency was not affected.

Table 3.9.1.1-2: Mean body weight (g) in male and female (m/f) rats (including satellite groups)

Dose level **Week**	**0 ppm**	**200 ppm**	**800 ppm**	**4000 ppm**
0 (start)	91 / 79	91 / 79	91 / 79	90 / 79
12	339 / 193	336 / 192	336 / 194	326***/190*
26	411 / 220	410 / 218	404*/ 219	388***/210***

52	459 / 249	460 / 245	454 / 246	424***/228***
78	479 / 294	470 / 301	467 / 289	445***/255***
104	428 / 313	430 / 326	436 / 325	411 / 295

* statistically significant, p<0.05; ** p<0.01; *** p<0.001

Ophthalmoscopy: After 24 months, ophthalmoscopy revealed increased numbers of unilateral cataracts in males of the highest dose level (see Table 3.9.1.1-3 below).

Table 3.9.1.1-3: Occurrence of cataracts in male and female rats after 24 months of treatment

Dose level	**0 ppm**	**200 ppm**	**800 ppm**	**4000 ppm**
Males	7	1*	4	18*
Females	1	2	2	5

* statistically significant, p<0.05

Hematology, Clinical chemistry. Urinalvsis: Hematological examination showed a prolongation of prothrombin time (PT) and partial thromboplastin time (PTT) in males of the 4000 ppm group after six and twelve months. In addition, PTT was prolonged in males of the 800 ppm group after 12 months. At the end of the study, a prolongation of PTT was noted in males of the 4000 ppm group. In females, this effect was considered equivocal (see Table 3.9.1.1-4). Erythrocyte parameters were within the normal ranges.

Table 3.9.1.1-4: Partial thromboplastin time (seconds) in male and female (m/f) rats after 6, 12, and 24 months of treatment

Dose level	**0 ppm**	**200 ppm**	**800 ppm**	**4000 ppm**
6 months	19.8/16.7	20.2/15.9	19.7/16.3	24.0***/17.4
12 months	17.8/17.3	19.1/17.4	20.2**/18.0*	22 .5***/18.6*
24 months	15.9/16.7	16.7/16.9	17.3/15.4*	18.7V17.3

* statistically significant, p<0.05; ** p<0.01; *** p<0.001

Biochemistry after six months revealed an increase of blood urea nitrogen in males of all dose groups and at 4000 ppm an increased A/G ratio. Cholesterol was decreased, and glucose and ASAT tended to decrease in males. Blood urea nitrogen was increased and ALAT was decreased in females of the 4000 ppm group. After 12 months, glucose, ASAT and sodium were decreased in males and A/G ratio, creatinine and blood urea nitrogen were increased in females of the highest dose group. At 800 ppm, blood urea nitrogen was increased in females. After 24 months, glucose and cholesterol were decreased in both sexes in the highest dose group. Urinalysis data after 6 and 12 months indicated an increased urinary output and a decrease in specific gravity and protein in both sexes of the 4000 ppm group. At 800 ppm, urine volume was increased coupled with a

decrease in specific gravity only at month 6. After 24 months the specific gravity was decreased without volume increase at 4000 ppm in both sexes and in females of the 800 ppm group.

Pathology: The absolute and relative kidney weight was increased in both sexes at 4000 ppm when examined after six months. Furthermore, decreased thyroid weight at 4000 ppm and 800 ppm as well as increased liver and decreased thymus weight in the 4000 ppm group were observed in females at this time. After 12 months, an increased absolute and relative kidney weight was noted at 4000 ppm in both sexes. The absolute and relative thyroid weight was decreased at 4000 ppm and 800 ppm in males. After 24 months, absolute and relative liver and spleen weights were decreased in males receiving 4000 ppm. At 800 ppm, relative liver weight was decreased in males. In females of the 4000 ppm group, relative kidney and the absolute and relative adrenal weight (left adrenal only) were increased. Necropsy findings of animals sacrificed at month 6 and 12 revealed no test substance-related changes. In all rats which died from month 13 onwards or were sacrificed at the end of the study, no substance-related tumors were noted. No other treatment-related changes were noted in female rats in all dose groups and in male rats up to the mid dose level of 800 ppm. In males of the 4000 ppm group, lesions of eye ball and retinal degeneration were observed in association with cataract at the end of the study. In addition there was evidence of atrophy of the optic nerve. However, the incidence of focal retinal atrophy was decreased in this group.

Conclusion

The administration of bentazone led to a reduction of the body weight gain at 4000 ppm. Although histopathology revealed no substance-induced changes, indications for an impairment of the liver and kidney function were noted at 800 and 4000 ppm by changes in clinical chemistry and urinalysis parameters and by increased organ weights as well as by an increased water consumption at the top dose level. Blood coagulation was affected at 800 and 4000 ppm. This finding is in agreement with the results of subacute and subchronic studies with bentazone. The decreased organ weights were assessed to be related to the decreased body weights. Under the conditions of this study, **the NOEL for chronic toxicity was found to be 200 ppm equivalent to 9 mg/kg bw for male and female animals** (derived from substance intake of the 104 weeks group). No carcinogenic effect was observed in this study.

This study served as basis for calculation of the ADI.

Retinal changes and cataracts are frequent, age-induced manifestations in this strain of rats with a widely variable incidence. It should be mentioned that all except one of the cataracts observed were unilateral. In case of a substance-related cataractogenic effect, bilateral cataracts would be expected. In a reexamination of the abovementioned clinical and pathological findings [Anonymous 1985 (Doc No 85/0442); Anonymous 1986 (Doc No 86/0438)], significant differences in the incidence of retinal degeneration and atrophy between treatment and control animals were not detected when these findings were regarded as one type of lesion.

Therefore, it is not likely that the ocular changes were substance-induced effects. The toxicological significance of the degenerative changes in the optic nerve remains equivocal, in particular, since a detailed examination revealing atrophy was confined to high dose males [Anonymous 1986 (Doc No 86/0438] . Furthermore, it should be taken into account that such eye findings were not noted in any other long-term or subchronic study with bentazone.

3.9.1.2 Study 2 – 2 year rat

Anonymous (1974) (Doc. No. 74/004)

Two year chronic oral toxicity study of Bentazone (BAS 351-H) in rats; BASF Reg. Doc. No. 74/004

Testing facility: Cannon Laboratories, Inc., Reading, USA; experimental work completed: 1974

Previous evaluation: in original DAR

Material and methods

Test method: This study was performed prior to implementation of specific test guidelines. The study is not in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.

GLP: No. The study was performed prior to implementation of GLP guidelines.

Acceptance: With respect to the year when it was performed, the study is considered acceptable although there were some reporting deficiencies.

Test system: Male and female Sprague-Dawley rats (50 per sex and dose group; source: not available) were administered bentazone technical (batch no. and purity not submitted) for two years at dose levels of 0; 100; 350 and 1600 ppm in the diet. The animals were housed singly under controlled conditions and received standardized diet and water ad libitum. The test substance/feed preparations were prepared monthly. Clinical observations were made up daily. Feed consumption was recorded weekly for the first 2 months and then every 2 weeks. Body weight was determined weekly for the first 2 months and monthly thereafter. Ophthalmoscopy was carried out prior to the beginning of the study and after 6, 9, 12, 18 and 24 months. Hematological, clinicochemical and urinalysis parameters were determined in 5 male and 5 female animals of each dose group after 3, 6, 9 and 12 months and in 10 male and 10 female animals of each test group after 18 and 24 months. After 12 months, 5 male and 5 female animals of each test group were sacrificed and necropsied. All the sacrificed animals were subjected to a gross-pathological examination. All sacrificed animals of the control group and of the highest dose group and 10 male and 10 female animals of each of the other dose groups were examined histopathologically.

Statistics: Various methods were applied including Bartlett's test for homogeneity of variances, analysis of variance, Duncan's multiple range test and Wilcoxon or Mann-Whitney rank sum test.

Results

Analysis: Analysis of the diet did not reveal any changes in the concentration after repelletizing or after one month of storage (not further specified).

Substance intake: The concentrations of 100; 350 and 1600 ppm corresponded to substance intakes of 5, 17 and 76 mg/kg bw per day (means for males and females).

General observations: Mortality was not affected by the substance administration. Statistical evaluation of body weight revealed a significant decrease at 1600 ppm in both sexes due to a diminished body weight gain in the second year of the study. Similarly, mean food consumption was reduced at the top dose level in the second year.

Table 3.9.1.2-1: Mean body weight (g) in male and female (m/f) rats

Dose level Week	**0 ppm**	**100 ppm**	**350 ppm**	**1600 ppm**
0 (start)	73.1 / 62.0	70.5 / 62.3-	73.3 / 61.6	71.0 / 60.9
24	466.2 / 291.5	470.5 / 306.0	467.7 / 297.7	476.1 / 304.2
52	528.9 / 366.2	510.2 / 347.8	521.2 / 334.1	520.6 / 366.8
72	540.9 / 371.2	520.2 / 360.0	550.2 / 340.3	490.3* / 358.9
96	544.9 / 385.1	524.9 / 372.5	566.6 / 355.0	484.1* / 350.2*

* statistically significant, $p<0.01$

Hematology. Clinical chemistry. Ophthalmoscopy: No treatment-related findings were noted. However, blood coagulation parameters were not investigated.

Pathology: At 1600 ppm, the following organ weights were increased: kidney, liver and spleen in both sexes, brain and heart in females. The organ to body weight ratio of kidney, liver and spleen was increased in both sexes. Female animals exhibited an increased brain- and heart-to-body weight ratio. No statistical significance was shown in any of the other three dose groups. Tumors that occurred were examined histologically and did not reveal any signs of malignancy. Statistical analysis of tumor incidence did not reveal any significance among the groups tested. No substance-induced histopathological changes were noted.

Conclusion

Under the conditions of this study, **the NOEL for chronic toxicity is 350 ppm equivalent to 17 mg/kg bw** for male and female animals. This value is based on decreased food consumption and body weight gain and increased organ weights at the highest dose level. The results of this study suggest that bentazone has no carcinogenic potential.

3.9.1.3 Study 3 – 2 year carcinogenicity mouse

Anonymous 1985 (Doc. No. 85/432)

Studies on the 24-month chronic toxicity of bentazone Reg. No. 51 929 (ZNT No. 81/273) in mice; BASF Reg. Doc. No. 85/432

Testing facility: date of experimental work completed: 23 November 1982

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed according to OECD guideline 453 and is in compliance with the demands of Directive 87/302/EEC, May 30, 1987.

Deviations: None.

GLP: Yes. When the study was performed, GLP was not compulsory. However, it is stated in the report that the study was run according to the principles of GLP.

Acceptance: The study is considered acceptable.

Test system: Male and female CRJ:B6C3Fl mice (source: Charles River Japan Co., Ltd.) were administered bentazone (purity: 93.9%; batch no. N 169) at dose levels of 0; 100; 400 and 2000 ppm as admixture in the diet. For the main study (24 months), control and dose groups consisted of 50 animals per sex each. 10 additional mice per sex and dose level were included in satellite groups for interim kill after 6 and 12 months. During the administration period, two animals per cage were housed under controlled conditions with free access to standardized diet and tap water. The test substance/feed preparations were prepared monthly. Clinical observations and mortalities were recorded twice daily. Palpation was done on skin and abdominal organs once a week. Body weight was determined weekly. Food and water consumption were recorded daily. Ophthalmoscopy, urinalysis, hematological and clinicochemical examinations were carried out after 6, 12 and 24 months. Gross-pathological and histopathological examinations were performed at sacrifice after 6, 12 and 24 months.

Statistics: Student's t-test. Fisher's direct computation for probability was applied to assess the incidence of clinical signs and pathological findings.

Results

Analysis: The analysis of the actual dietary concentrations confirmed the intended dose levels (100 ppm: 102.3%; 400 ppm: 102.4%; 2000 ppm: 98.7% of theoretical values).

Substance intake: The mean substance intake is shown in Table 3.9.1.3-1.

Table 3.9.1.3-1: Mean substance intake

Dose level (ppm)	**Mean substance intake (mg/kg bw)**	
	Males	**Females**

100	12	12
400	47	48
2000	242	275

General observations: No clinical signs were observed during the 24-month study period. The mortality rate of the treated animals was not significantly different from that of the control. Male animals of the 2000 ppm group showed a slight but statistically significant, transient suppression of their body weight. There was no significant difference between the control and treated animals in food consumption, food efficiency and water consumption throughout the study period. Ophthalmoscopy revealed no substance-induced changes in any of the treated animals after 6, 12 and 24 months.

Hematology. Clinical chemistry, Urinalvsis: Urinalysis data indicated an increased urinary specific gravity in male mice of the 400 ppm and 2000 ppm groups after 12 months. Hematology revealed intermittendly reduced RBC and increased MCV and MCH values in female mice of the treated groups after 6 months and reduced WBC values after 12 months. However, since the incidences of these changes varied at the different examinations and no permanent effects could be recorded, it is very unlikely that they were substance-related. At 400 and 2000 ppm, male mice exhibited prolonged PT values after 24 months (see Table 3.9.1.3-2). Clinical chemistry revealed no substance-related abnormalities in male and female mice of the treated groups after 6, 12 and 24 months.

Table 3.9.1.3-2: Blood coagulation parameters in male mice after 24 months of treatment

Dose level	**0 ppm**	**100 ppm**	**400 ppm**	**1600 ppm**
Prothrombin time (PT) in seconds	13.5	13.0	15.2*	21.5*
Partial thromboplastin time (PTT) in seconds	22.8	20.6	25.4	26.7

* statistically significant, $p<0.05$

Pathology: Absolute and relative organ weights were not affected by the test substance administration. Gross pathological examination revealed various changes in the liver, spleen and thymus in male and female mice of each group at the end of the study, but there were no lesions considered to be clearly due to test substance administration. The only non-neoplastic finding suspected to be due to test substance administration was an increased calcification of the testicular tunica albuginea in males of the two upper dose groups (see Table 3.9.1.3-3).

Table 3.9.1.3-3: Number of male mice exhibiting testicular calcification after 24 months (n=50/group)

Dose level	0 ppm	100 ppm	400 ppm	1600 ppm
Calcification	2	6	12**	3 5** *

** statistically significant, p<0.01; *** p<0.001

Histopathological examinations revealed several tumors. With the exception of certain liver tumors, each type of tumor found in the treated groups did not differ significantly from the controls. In males, the number of hepatocellular carcinoma was significantly increased at 400 and 1600 ppm among animals which died intercurrently or were killed in extremis. But this was not true for the animals of the same groups killed by design. However, the total number of liver cell carcinoma was increased at least at the top dose level. In contrast, the incidence of neoplastic nodules in the liver was significantly decreased in the highest dose group. When the combined incidence of these two types of liver tumors was regarded there were no significant differences between the groups and no dose-related increase. In female animals of the highest dose group, an increased number of gray nodules was observed in the liver (see Table 3.9.1.3-4). For evaluation purposes, all the liver findings were reexamined as described and discussed below.

Table 3.9.1.3-4: Summary of neoplastic liver findings in male and female mice (n=50 per sex and dose group) in the main study (24 months)

Dose level	0 ppm	100 ppm	400 ppm	1600 ppm
Carcinoma in males dying or being killed inter-currently	1/14	2/14	6*/15	15***/21
Carcinoma in males killed by design	5/36	8/36	7/35	6/29
Neoplastic nodules in males dying or being killed intercurrently	4/14	3/14	6/15	0*/21
Neoplastic nodules in males killed by design	16/36	15/36	16/35	8/29
Combined incidence, males (total)	26/50	28/50	35/50	29/50
Neoplastic nodules in females (total)	3/50	4/50	4/50	ll*/50

* statistically significant, p<0.05; *** p<0.001

Conclusion

The administration of bentazone to male animals of the high dose group led to a slight but statistically significant and transient suppression of their body weight gain. An impairment of blood coagulation was observed in male animals of the two upper dose groups and was in agreement with similar findings in the

short-term toxicity studies in mice and in a chronic study in rats (section 3.9.1.1). The gross-pathological examination showed various lesions in the liver, spleen and thymus in animals of all the treatment groups and the control group. These lesions occurred in some cases significantly more frequently only in the two intermediate dose groups and showed no dose-response relationship. They were assessed as being age-induced and not being related to the administration of the test substance. The histopathological examination revealed increased testicular calcification in male mice of the two upper dose groups. This change is a very common lesion in aged mice including the strain used in this study. In view of historical control data, this finding was rather considered to be of an age-induced spontaneous nature. As stated by an independent expert [Anonymous 1985 (Doc No 85/440)], the degree of calcification was mostly very slight. There was no evidence of another adverse effect of bentazone on testes or on male reproductive performance obtained in any study on this compound. However, the toxicological significance of this change remains equivocal. It should be mentioned that an increased incidence of testicular calcification was not observed in any other long-term study with bentazone.

The histopathological examination of the liver changes was carried out by the testing facility and, due to difficulties in the interpretation, subsequently by external experts [Anonymous 1985 (Doc No 85/0442); Anonymous 1985 (Doc No 85/431); Anonymous 1987 (Doc No 87/0139); Anonymous 1988 (Doc No 88/0483)]. The results of these examinations revealed considerable differences. Thus, while the study authors were noting a total incidence of 21 hepatocarcinomas in high dose males, Anonymous 1985 (Doc No 85/0442) found only 10. In contrast, he diagnosed a higher number of carcinomas in the control group (9 instead of 6). In re-examination at the testing facility itself, these latter results were confirmed (Anonymous 1985 (Doc No 85/431) . The apparent difference could be at least partly due to different diagnostic criteria applied and to post mortem artefacts in the mice found dead. In the third expert statement (Anonymous 1987 (Doc No 87/0139; Anonymous 1988 (Doc No 88/0483)) including blind reading of all slides by three pathologists, an additional discrimination between adenoma and focal nodular hyperplasia has been made. No significant increase in neither these two lesions nor in carcinomas was noted in males. In females, no increase in adenomas or carcinomas was observed. In spite of these contradictory results between the original study report and the subsequent expert statements, it can be assumed that male mice indeed exhibited a significantly increased number of carcinomas of the liver at 1600 ppm in this study; however, the incidence of nodules (e.g. hyperplasia or adenoma) was lower. The development of carcinomas was preceded by the so-called neoplastic nodules (Anonymous 1985 (Doc No 85/431) . Therefore, it seems reasonably to take into account also the combined incidence of these lesions. When regarded together, nodules and carcinomas of the liver in the treated animals including the highest dose group were not detected significantly more frequently than in control animals.

The pathologists involved agreed that hepatic nodules (including all non-carcinomatous nodular changes including adenomas) or at least nodular hyperplasia occurred significantly more frequently in female mice of

the highest dose groups. However, the historical background incidence of this lesion in mice of this strain is rather high (Anonymous 1985 (Doc No 85/0442). The number of carcinomas in the liver did not differ from control values. Thus, no evidence of increasing malignancy was obtained in females.
The experts came to the conclusion that no carcinogenic potential can be assigned to bentazone:
"The overall conclusion is that while bentazone may have increased slightly proliferative lesions in the liver of the female mice, it did not have a carcinogenic effect as evidenced by the numbers of hepatocellular carcinomas in the mice of the test groups." (Anonymous 1987a (Doc No 87/0139) In the meantime, both the U.S. EPA [Anonymous 1989 (Doc No 1989/10485)] and the Californian registration authorities [Anonymous, 1987] have endorsed this assessment. The histopathological examination of the other organs did not reveal any tumor incidences that were significantly increased as compared with control values. **The NOEL for chronic toxicity was 100 ppm or 12 mg/kg bw for males and females.**

3.9.1.4 Study 4 – 18 month carcinogenicity mouse

Anonymous 1974 (Doc. No. 74/041)
18 month chronic oral toxicity study of BAS 3 51-H in mice; BASF Reg. Doc. No. 74/041
Testing facility: date of test report: 13 November 1974
Previous evaluation: in original DAR

Material and methods
Test method: The study was performed prior to implementation of specific test guidelines. The study is not in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.
GLP: No. The study was performed prior to implementation of GLP guidelines.
Acceptance: The study is considered supplementary only since it does not comply to current standards and due to the high mortality in all groups. In addition, hematological and clinical chemistry parameters were not investigated.
Test system: Male and female Swiss Webster mice (source: not available) were administered bentazone technical (batch no. not available) at dose levels of 0; 100; 350 and 1600 ppm as admixture in the diet for 18 months. Each group consisted of 50 animals per sex. Five mice per sex and dose group were subjected to interim sacrifice after 12 months. The animals were housed four per cage under controlled conditions and received standardized diet and water ad libitum. The test substance/feed preparations were prepared monthly. Clinical observations were made up daily. Body weight was determined weekly for the first 8 weeks and then monthly. Food consumption was recorded daily. Ophthalmoscopy was performed prior to the start of the study and after 6, 9, 12 and 18 months. All animals were examined gross-pathologically. 10 males and 10 females of the low and mid dose and all animals from the high dose and control group were examined histopathologically.

Statistics: Calculation of body weight, food consumption, tumor incidence, organ weight and organ to body weight ratio data was performed by means of various methods including Bartlett's test for homogeneity of variances, analysis of variance, Duncan's multiple range test and Wilcoxon or Mann-Whitney rank sum test.

Results

Analysis: Analysis of the diet did not reveal any changes in the concentration after repelletizing or after one month of storage (not further specified).

Substance intake: The concentrations of 100; 350 and 1600 ppm corresponded to substance intakes of 15; 52 and 237 mg/kg bw per day (means for males and females).

General observation: More than 50% of the animals in the test and control groups died in the course of the study. Female animals in the highest test group exhibited significantly reduced food consumption and lower body weights. Male mice also showed a decrease in body weight and food consumption after 18 months but statistical significance was not reached.

Pathology: Substance-induced gross-pathological changes were not noted. At terminal sacrifice, mean absolute brain and liver weight in females of the 1600 ppm group were increased. In males, the absolute spleen weight was decreased. At this top dose level, the following relative organ weights were increased: brain and liver in both sexes, kidney and heart in females. In males, the relative spleen weight was decreased. No substance-induced histological changes were found. No differences in the tumor incidence were observed between treated and control groups.

Conclusion

The administration of bentazone led to an impairment of body weight gain and food consumption as well as to organ weight changes at the highest dose level. The mortality rate was above 50% in all groups and therefore no conclusive assessment of the observed effects is possible.

Under the conditions of this study, **the NOEL is 350 ppm (corresponding to 52 mg/kg bw) for male and female animals.** Although the study mentioned above no longer complies with current guideline requirements, the results of this study suggest that bentazone has no carcinogenic potential.

3.9.1.5 Study 5 –82-95 week carcinogenicity mouse

Anonymous 1978 (Doc. No. 78/034)

Tumorigenicity of bentazone acid to mice in long term dietary administration; BASF Reg. Doc. No. 78/034

Testing facility: experimental work completed: 1977

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed prior to implementation of specific test guidelines. The study is not in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.

GLP: No. The study was performed prior to implementation of GLP guidelines.

Acceptance: The study is considered supplementary since it does not comply with current guideline requirements. In particular, the number of animals per group was too low when the study commenced. Even at 78 weeks, mortality exceeded 50% in one group.

Test system: Bentazone technical (batch p. 195.75) was administered to male and female CFLP mice {source: Anglia Laboratory Animals, Alconbury, England) at dose levels of 0; 100; 350 and 1600 ppm as admixture in the diet. Dose groups consisted of 40 mice per sex. When approximately 25% survival had been attained among a control or treated group (except the top dose group), all animals of that sex were killed. Therefore, all surviving male mice receiving 1600 ppm were sacrificed after 82 weeks and all the other male groups were terminated after 88 weeks of treatment. The female groups were maintained up to 95 weeks. The animals were housed 4 to a cage under controlled conditions and had free access to tap water and standardized diet. The test substance/feed preparations were prepared weekly. Clinical observations were made up daily. Food consumption was recorded weekly and body weight was determined weekly for the first 3 months and thereafter every two weeks. Clinicochemical and hematological examinations were not carried out. At termination, all surviving animals were sacrificed and examined gross-pathologically. Gross lesions as well as liver, spleen, lymph nodes, adrenals, thyroid, ovaries and pineal body were examined histopathologically.

Statistics: Mortality rates were compared between the groups using Stratified Contingency tables. Student's t-test was applied to assess the significance of intergroup differences in body weight, food and water intake data.

Results

Analysis: The dietary concentrations were analysed by the study sponsor and the results are compiled in Table 3.9.1.5-1. With respect to an accepted deviation of max. 10%, most of the analyses confirmed the intended concentrations.

Table 3.9.1.5-1: Results of actual dietary concentrations analysis

Nominal	**Analysed concentration (ppm)**				
	Week 1	**Week 26**	**Week 52**	**Week 78**	**Week 94**
100	89	98	86	84	90
350	325	290	309	284	314
1600	1528	1430	1401	1240	1320

Substance intake: The mean substance intake is shown in the following table:

Table 3.9.1.5-2: Mean substance intake

Dose level (ppm)	Mean substance intake (mg/kg bw)	
	Males	**Females**
100	8.4	9.5
350	29.7	34.3
1 600	138.4	152.9

General observation: No substance-induced findings were found in clinical parameters. The number of deaths among treated mice was similar to that of the controls, with the exception of males receiving 1600 ppm, where there was an increased incidence of mortality during weeks 79 to 82. At week 78 (the required duration of carcinogenicity study in mice), survival in the male animals had declined to 27, 21, 30 and 23 mice in the control, 100, 350 and 1600 ppm groups, respectively. Among females, 29, 31, 26 and 18 animals were still alive in the respective groups. Thus, at 1600 ppm, mortality had reached 55% in females at this time. However, since no macroscopic and microscopic changes which could be attributed to treatment were noted in the animals dying intercurrently, the higher mortality rate was not assessed as being substance-induced. Food consumption and body weight gain of treated animals were similar to those of control animals.

Pathology: No substance-induced changes were found in any gross-pathological or histopathological parameters.

Conclusion

Under the conditions of this study, **no carcinogenic effect was suggested and the NOEL was 1600 ppm corresponding to about 146 mg/kg bw (mean for both sexes).**

However, the scientific value of this outcome is limited due to the insufficient number of animals on study and the high overall mortality.

3.9.2 Human data

No data.

3.9.3 In vitro data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

No data.

3.9.4 Other data (e.g. studies on mechanism of action)

No data.

3.10 Reproductive toxicity

3.10.1 Animal data

Submission of studies for the renewal process, and not included in the DAR dated September 1996:
No new studies were submitted. In the European Commision Final Review Report of bentazone the effect of pup weight (Anonymous 1989 (Doc. No. 89/0068), section 3.10.1.2) was considered to be related to parental toxicity based on the haematological and clinical chemistry alterations at 800 ppm in the chronic rat study. In the most recent evaluation of the same data by EPA this conclusion was not shared and bentazone was concluded to affect the offspring at non parentally toxic doses. Therefore, the notifier has provided a re-evaluation of the study, including historical control data, a check for maternal toxicity and litter size effect, to show that reduced pup weight gain was predominantly seen in dams with markedly reduced food consumption. Additional information on the study is included in section 3.10.1.2.

3.10.1.1 Study 1 – 3- generation study in rat

Anonymous 1973 (Doc. No. 73/010)
Chronic oral toxicity of bentazone in a reproduction study covering three generations of Sprague-Dawley rats; BASF Reg. Doc. No. 73/010
Testing facility: experimental work completed: 06/1973
Previous evaluation: in original DAR

Material and methods
Test method: This study was performed prior to implementation of specific test guidelines. Its major part is not in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.
GLP: No. The study was performed prior to implementation of GLP guidelines.
Acceptance: The study is considered supplementary only since information regarding origin and purity of the test compound is lacking. In addition, it should be mentioned that a dose level causing systemic toxicity was not included for testing.
Test system: Bentazone technical (batch no. not available) was administered to three generations (P, F1, F2) of male and female Sprague-Dawley rats (source: Ivanovas, Kifilegg, Germany) as admixture in the diet. Dose levels were 0; 20; 60 and 180 ppm. Twenty rats per sex and dose group were used to form the respective parental generations. Initially, all rats were housed 3 per cage under controlled conditions. For mating, one male and one female were placed together during the 12-hour dark period for seven days. Once copulation had occurred, the females were separated and kept alone. The animals had free access to

standardized diet and drinking water throughout the study. The test substance/feed preparation were made up daily.

Breeding program: After pre-treatment of 8 to 18 weeks, the P-animals were mated. The pups of the first litter (F1a) were reared until they were 4 weeks old and then sacrificed and necropsied. The parental animals were mated again and at an age of 4 weeks, 20 animals per dose group and sex were selected from the pups of the second litter and reared while being given further treatment. The remaining pups were sacrificed and necropsied. At an age of 18 to 29 weeks, these F1b animals were mated twice and the same procedure was carried out with the F2 pups as with the F1 pups. From the F3 pups, twenty animals per dose and sex were observed for further five weeks after weaning being sacrificed at an age of nine weeks.

Clinical observations, food and drinking water consumption were recorded daily. Body weight was determined weekly. The gestation rate, litter size, weight at birth and weight gain of the pups, survival rate of the pups and incidence of anomalies were used as parameters for determining any effects on reproduction. Before termination, ophthalmoscopy and a hearing test were carried out. All animals were examined gross-pathologically. 10 animals per sex and dose of the F2 and F3 generations (the latter at an age of nine weeks as described above) were subjected to histopathological examination.

Statistics: Student's t-test.

Results

Analysis: Not specified.

Substance intake: The mean substance intake is shown in the following table:

Table 3.10.1.1-1: Mean substance intake

Dose level (ppm)	**Mean substance intake (mg/kg bw/d)**	
	Males	**Females**
P generation		
20	1.6	2.5
60	4.7	7.3
180	15.0	21.9
F1 generation		
20	1.6	2.4
60	4.5	7.2
180	14.1	21.5
F2 generation		
20	1.6	2.1
60	4.8	6.6
180	14.2	20.5
F3 generation		
20	2.2	2.0
60	6.4	6.4

180	19.4	19.8

General observations: No clinical signs were observed and the body weight remained unaffected.
Reproduction parameters: Fertility and rearing behavior of the animals were not affected. The development of the pups was comparable in all the groups.
Pathology: No substance-induced gross-pathological or histopathological changes occurred.

Conclusion

Under the conditions of this study, **the NOEL for both parental toxicity as well as reproductive and developmental toxicity was 180 ppm corresponding to about 18 mg/kg bw/day.**

3.10.1.2 Study 2 – 2-generation study in rat

Anonymous 1989 (Doc. No. 89/0068)

Two-generation reproduction study with bentazone technical (ZST No.:86/48) in the rat; BASF Reg. Doc. No. 89/0068
Testing facility: date of experimental work completed: 1 October 1987
Previous evaluation: in original DAR

Material and methods

Test method: The study was performed according to OECD guideline 416; EPA/FIFRA, Subdivision F, guideline 83-4 and Japan MAFF guidance no. 4200 and is, therefore, in compliance with the demands of Directive 87/302/EEC, May 30, 1987.
Deviations: None.
GLP: Yes. When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP. A formal GLP Compliance and a Quality Assuarance Statement are included in the report.
Acceptance: The study is considered acceptable.
Test system: Male and female Wistar/HAN rats (source: Madoerin AG, Fuellinsdorf, Switzerland) were administered bentazone (purity: 97.8%, batch no. N 187) for two generations at dose levels of 0; 200; 800 and 3200 ppm as admixture in the diet. Experimental groups (parental P and F1 generations) consisted of 25 rats per sex and dose. The animals were housed singly under controlled conditions and received standardized diet and tap water ad libitum. The test substance/feed preparations were freshly prepared at least every 2 weeks. During the pairing period, they were housed one male/one female in pairing cages. Breeding program: The animals were mated after a pretreatment period of 70 days. On day 4 after parturition, the litters were culled to 8 pups per litter. 25 male and 25 female pups were selected for further treatment on day 21 after parturition, the remaining pups and parental animals being sacrificed and necropsied. The F1

animals selected were administered the test substance for 123 days and then mated. The size of the litters was again reduced on day 4 after parturition. All the F2 pups and the F1 parental animals were sacrificed on day 21 after parturition and necropsied. Mortality and clinical observations were checked twice daily. Body weight and feed consumption were recorded weekly. Ovarial cycle, conception rate, post-implantation losses, duration of gestation, litter size, lactation, rearing behavior, weight at birth and body weight gain of the pups and their mortality up to day 4 or 21 after parturition were assessed. All animals were examined gross-pathologically. All mated animals of the F0 and F1 generations of the highest dose and control group and all animals that•died during the study were subjected to a histopathological examination.
Statistics: Dunnett test for body weight and food consumption; Kruskall-Wallis test or Fisher's Exact test for reproduction data.

Results

Analysis: The analytical examinations showed the stability in the diet for 21 days. The analysis of the mean dietary concentrations confirmed the intended dose levels (200 ppm: 99.2% of theoretical value; 800 ppm: 95.3% of theoretical value; 3200 ppm: 102.6% of theoretical value). The homogeneity of the substance/diet mixtures was also confirmed.
Substance intake: The mean substance intake is shown in the following table:

Table 3.10.1.2-1: Mean substance intake

Dose level (ppm)	**Mean substance intake (mg/kg bw/d)**	
	Males	**Females**
P generation (prepairing period, day 1 to day 70)		
200	14.8	17.0
800	58.5	66.9
3200	238	268.9
P generation (after pairing, day 1 to day 21/22)		
200	10.3	14.7
800	40.7	60.7
3200	164.3	246.7
P generation (lactation period, day 1 to day 14)		
200	-	29.7
800	-	111.0
3200	-	472.7
F 1 generation (prepairing period, day 1 to day 123)		
200	13.7	15.9
800	56.9	64.4
3200	227	261.6
F 1 generation (after pairing, day 1 to day 25, males/gestation priod, day 0 to day 21, females)		
200	10.0	14.3
800	40.8	59.3

3200	168.3	238.7
F 1 generation (lactating period, day 1 to day 14)		
200	-	29.0
800	-	121.3
3200	-	492.0

Parental findings: Decreased mean body weight was observed in the 3200 ppm group in the F1 males during the prepairing and postpairing periods and in P and F1 females during all periods. The differences from the control group were statistically significant at most time points. Although the mean value of maternal feed intake at the mid dose did not differ statistically significant from the control groups, the individual analysis of the data did reveal clear signs of decreased food consumption in particular on PND 1-4 (Table 3.10.1.2-2). Taking only those dams into account which had a total litter loss or whose pups showed reduced pup weights over the period of PND 4-7 which are #152, 155, 156, 159, 164, 167, 171, 172 and 175, the mean food consumption between PND 1 and 4 is 14.2 ± 9.6 g (see Table 3.10.1.2-2). This is a significant reduction of about 52% compared to the control and the body weight gain was reduced to 4.8 g between PND 1 and 4, which is only 29 % of the concurrent control.

A further focusing on only those dams that showed a body weight reduction based on maternal toxicity and not those that had reduced pup weight based on high litter size would additionally eliminate dam # 152 and #172. This leads to a reduced food consumption of 10.4 ± 6.7 g (38.4% of control) and the mean maternal body weight gain to -1.7 g between PND1 and 4. This clearly demonstrates a pronounced maternal toxicity at 800 ppm based on a significantly reduced food intake and weight gain values.

Table 3.10.1.2-2 P-generation nursing the F1 pups: 800 ppm groups; Individual maternal food consumption data and body weight respective Body weight gain of the F0 dams and the corresponding F1 litter body weight means and litter size.

800 ppm Dam #	Food consumption [g/animal/day]								BW [g]	BWG [g]	Mean Pup weight [g]		
	Gestation		Lactation						maternal				
	Days 1-21	Dev.to Control	Days 1-4	Dev.to Control	Days 4-7	Dev.to Control	Days 7-14	Dev.to Control	Days 1	Days 1-4	Litter size	PND 1	PN D4
152	60	99%	25	92%	41	105%	47	92%	218	33	14	5.1	6.6
155	60	99%	5	18%	33	84%	49	96%	244	-9	13	5.4	4.8
156	67	111%	19	70%	35	89%	47	92%	253	7	15	4.9	6.3
159	70	116%	3	11%	-	-	-	-	258	-5	14	5.7	-
164	67	111%	10	37%	-	-	-	-	279	-26	5	5.5	-
167	62	103%	14	51%	32	82%	37	73%	263	4	9	5.8	5.5
171	65	108%	4	15%	28	71%	52	102%	261	-14	13	5.2	5.6
172	-	-	30	110%	42	107%	55	108%	259	22	15	4.9	6.8
175	55	91%	18	66%	62	158%	47	92%	224	31	11	5.0	6.0
Mean	63.3	105%	**14.2**	**52.3%**	39.0	99%	47.7	94%	**251.0**	**4.8**		**5.3**	**5.9**
								Dev. to control	**102%**	**29%**		**88%**	**69 %**

Under exclusion of #152 and # 172 which showed reduced pup weight despite normal food intake													
Mean	63.7	105%	**10.4**	**38.4%**	38.0	97%	46.4	91%	**254.6**	**-1.7**		**5.3**	**5.6**
								Dev. to control	**103%**	**-10%**		**90%**	**66 %**

Reproductive parameters/Pup findings: For the animals of both generations the following parameters were not influenced by treatment with bentazone when compared with their respective control groups: viability, behavior and general appearance, food consumption, duration of gestation, fertility index, post-implantation losses, parturition, quantity and quality of progeny, postnatal loss up to day four post partum and breeding loss from day four post partum (after culling) up to day 21 post partum, lactation and nursing, development and behavior of pups, incidence of malformations and/or anomalies. The only pup finding was a decreased mean body weight of F1 and F2 pups in the 800 ppm and 3200 ppm groups, statistically significant at most time points (as stated by the applicant). In particular, body weight development was retarded. All mean pup weights in the F1 generation of the mid and high dose group were significantly reduced compared to the concurrent control according to the statistical evaluation of the study report (Table 3.10.1.2-2). Historical control data were not reported but have now been made available (Anonymous 2011 (Doc No 2011/1145234); Anonymous 2011 (Doc. No. 2011/1248852). The mean pup weights were most affected in the mid dose at day 4 and 7 with a -11.9 % reduction compared to control at day 4 and -10.2% at day 7; both values were outside the historical control mean values (Table 3.10.1.2-4). The high dose group showed the peak at day 7 with a -8.8 % reduction vs. control. Furthermore, the pup weights of the high dose group were outside the historical control at day 4 with a -8.3 % reduction to the concurrent control. The later timepoints revealed the tendency of recovery and were additionally within the range of historical control means.

Table 3.10.1.2-2: Mean pup body weight (g) in the F1 litter (males/ females), statistical significances not indicated in the original report

Dose level	**0 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**
day 1	6.1/5.7	5.9/5.6	5.6/5.3	5.7/5.4
day 4	8.6/8.1	8.4/8.1	7.6/7.2	7.9/7.5
day 7	14.0/13 .4	13.7/13.1	12.6/12.0	12.8/12.1
day 14	28.9/28.3	29.2/28.5	27.9/26.8	27.4/26.3
day 21	46.0/44.5	47.0/45.6	43.9/42.3	44.3/42.5

Table 3.10.1.2-3: Mean pup body weight (g) in the F2 litter (males/ females), statistical significances not indicated in the original report

Dose level	**0 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**
day 1	6.1/5.9	6.2/5.8	6.1/5.7	6.3/5.9
day 4	9.2/9.0	8.9/8.4	9.2/8.5	8.8/8.4
day 7	15.0/14.3	14.6/14.0	14.8/13.8	14.2/13.7

day 14	31.6/30.4	30.9/30.1	30.7/29.4	29.0/27.9
day 21	51.9/49.6	51.5/49.6	49.9/47.4	47.4/45.6

Table 3.10.1.2-4: Comparison of mean pup weight with historical control data for the F1-generation

	F1 pups body weight during lactation [g]				**Historical control F1-generation (n=191 litters)**		
	Mean absolute values [g][1] (%Deviation to current control)				**Range of body weight means [g] (n=8)**		**Individual range [g] Mean ± SD**
Day	**0 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**	**Lowest**	**Highest**	
1	5.9	5.7 (-3.4)	**5.4 (-8.5)**	**5.5 (-6.8)**	5.4	6.1	5.8 ± 0.7
4	8.4	8.3 (-1.2)	***7.4 (-11.9)***	***7.7 (-8.3)***	7.9	8.8	8.3 ± 1.1
7	13.7	13.4 (-2.2)	***12.3 (-10.2)***	**12.5 (-8.8)**	12.5	14.2	13.3 ± 1.8
14	28.6	28.8 (0.7)	**27.4 (-4.2)**	**26.9 (-5.9)**	24.4	29.6	27.6 ± 3.4
21	45.3	46.2 (2.0)	**43.1 (-4.9)**	**43.5 (-4.0)**	36.9	47.4	43.3 ± 5.4
Mean Litter size	10.8	11.7	11.7	11.3	11.5		

1) Statistically significantly reduced means as mentioned in the study report are given in **bold**.
2) Historical control values as calculated from Historical control data (HCD) report Anonymous 2011 (Doc. No. 2011/1248852); Anonymous 2011 (Doc No 2011/1145234). The HCD is based on eight 2-generation studies, all conducted with the same strain of rats. The studies were performed at RCC Ltd. during the years 1985 to 1989 as dietary studies with a pre-pairing period of 56 to 70 days in the P generation and of at least 101 days in the F1 generation.

Italic marked values are outside the historical control values.

Table 3.10.1.2-5: Comparison of mean pup weight with historical control data for the F2-generation

	F2 pups body weight during lactation [g]				**Historical control F2-generation[2] (n=188 litters)**		
	Mean absolute values [g] [1]/ (%Deviation to current control)				**Range of body weight means [g] (n=8)**		**Individual range [g] Mean ± SD**
Day	**0 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**	**Lowest**	**Highest**	
1	6.0	6.0 (0)	5.9 (-1.7)	6.1 (+1.7)	5.7	6.1	6.0 ± 0.7

4	*9.1*	8.7 (-4.4)	**8.8 (-3.3)**	**8.6 (-5.5)**	8.4	9.0	8.9 ± 1.3
7	14.6	14.3 (-2.1)	14.3 (-2.1)	**14.0 (-4.1)**	13.2	14.7	14.2 ± 1.9
14	31.0	30.5 (-1.6)	**30.1 (-2.9)**	**28.5 (-8.1)**	26.0	31.3	29.5 ± 3.4
21	*50.7*	50.6 (-0.2)	**48.7 (-3.9)**	**46.5 (-8.3)**	42.3	50.5	47.9 ± 5.2
Mean Litter size	10.5	11.5	11.0	10.8	11.2		

1) Statistically significantly reduced means as mentioned in the study report are given in **bold**.
2) Historical control values as calculated from Historical control data report Anonymous 2011 (Doc. No. 2011/1248852); Anonymous 2011 (Doc No 2011/1145234). The HCD is based on eight 2-generation studies, all conduted with the same strain of rats. The studies were performed at RCC Ltd. during the years 1985 to 1989 as dietary studies with a pre-pairing period of 56 to 70 days in the P generation and of at least 101 days in the F1 generation.

Italic marked values are outside the historical control values.

A steady dose relationship is not evident in the F2 generation. The F2 generation is less affected than the F1 generation, showing statistically reduced mean pup body weights in the mid dose at PND 4, 14 and 21 and in the high dose from PND 4 to PND 21. All F2 pup body means of animals treated with bentazone are within the range of the historical control values. Whereas the low and mid dose groups are from PND7 onwards rather above the average historic mean value, the 3200 ppm group is slightly and throughout the whole dosing period below the mean historical control value. The concurrent control is rather high, for day 4 and 21 even above the top border of the historical control means.

In the original assessment the effect of litter size differences was not considered. The existence of an inverse relationship of pup body weight development and litter size at least until culling at PND 4 due to competition for maternal milk is intensively described in Agnish & Keller, 1997 [Fundam. Appl. Toxicol. 38, 2–6]. This characteristic is also seen in the HC data: the pre-culling data at PND 1 and 4 shows the typical inverse relationship between litter size and mean pup weight development, represented by a linear trend line with negative slope as indicated in Figure 3.10.1.2-1 for the F1 generation and Figure 3.10.1.2-2 for the F2 generation below.

Figure 3.10.1.2-1 Historical control data: Correlation of mean litter size and mean pup weight at PND 1 and 4 in the F1-pup generation

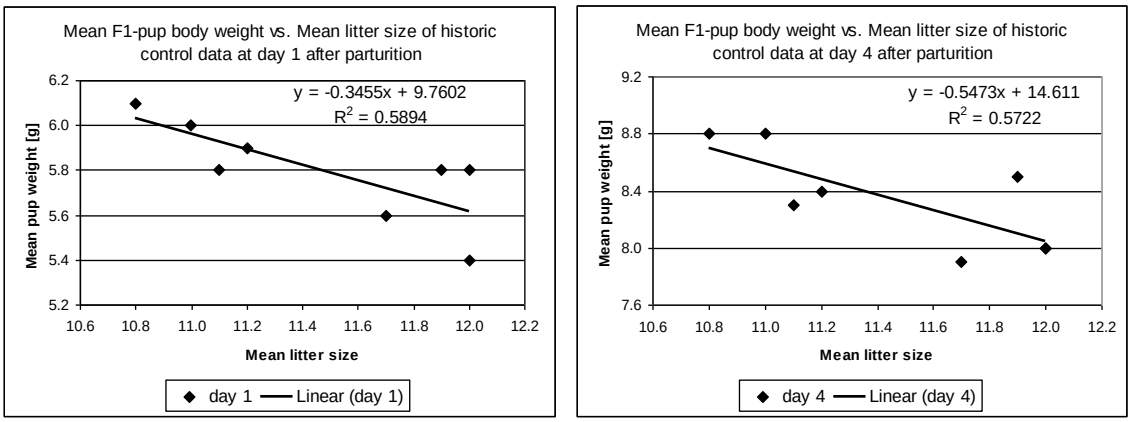
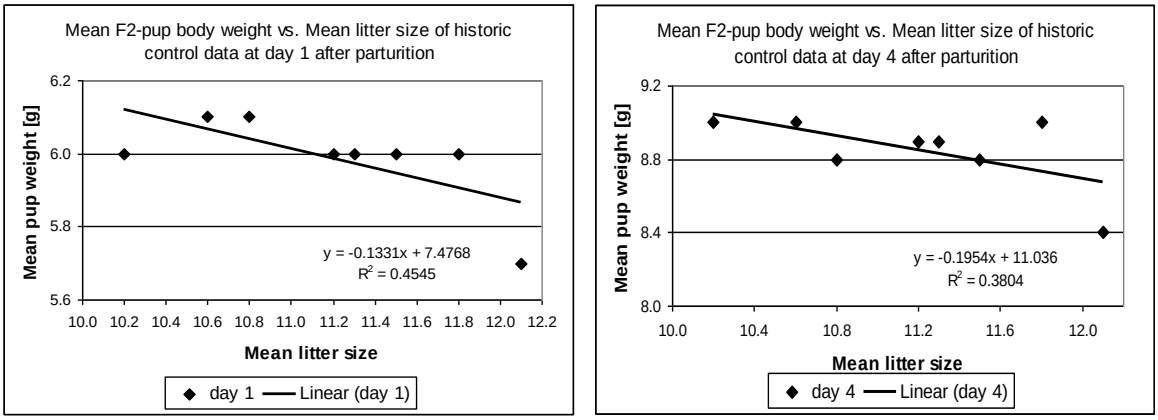


Figure 3.10.1.2-2 Historical control data: Correlation of mean litter size and mean pup weight at PND 1 and 4 in the F2-pup generation



Anonymous (2009, Doc No 2011/1262290) introduced an approach to standardize pup weight data for litter size effects by correction factors generated in a historical control cohort until the day of culling. This approach is described on the basis of individual data in the HC cohort to correct the individual data in the corresponding study. This approach was adopted to correct the mean values on the basis of the linear regression parameters generated in the Historical control data for day 1 and day 4, leading to normalized mean pup weights as indicated in the table below.

Table 3.10.1.2-6 Normalized mean pup body weights in the F1 generation

Day	**Mean pup body weight values [g] (% Deviation to respective control)**			
	F1 pups body weight during lactation [g]			
	0 ppm	**200 ppm**	**800 ppm**	**3200 ppm**
1	5.7	5.8 (2%)	5.5 (-3%)	5.4 (-4%)
4	8.0	8.4 (5%)	7.5 (-7%)	7.6 (-5%)

Further evidence that the pup weights are less substance affected than indicated in the original report is the comparison of the mean litter weights. The mean litter weights show at PND 1 no deviation to control and at PND 4 a reduction of 3 to 4% in the mid and high dose group.

Table 3.10.1.2-7 F1 mean litter weight at PND 1 and 4 [g]

Day	Mean values ± SD [g] / (%Deviation to current control) [1]			
	0 ppm	200 ppm	800 ppm	3200 ppm
1	63.7 ± 12.4	66.5 ± 10.2 (+4.4)	63.8 ± 13.4 (+ 0.1)	63.0 ± 11.2 (-1.1)
4	88.6 ± 14.9	96.1± 13.2 (+8.5)	85.7 ± 20.3 (-3.2)	85.1 ± 18.0 (-3.9)

1) Mean values and Deviation from the control [%] calculated from reported absolute values.

To summarize, there is no substance-induce effect on fetal body weigth on PND1 as shown by unaffected mean litter weight. The reduced pup weight at PND4 and 7 at 800 and 3200 ppm is unlikely to be directly caused by bentazone, but rather a consequence of maternal toxicity (manifested as reduced feed intake during PND1-4). Overall pup weight effects are correlated to higher litter size and/or to significant reduced maternal feed intake within the early lactation phase. Therefore, the relevant timeframe for NOAEL setting is PND1-4 and not the gestation phase.

Pathology: Gross and histopathological examination did not reveal evidence of treatment-related changes neither in the adults nor in the offspring of any generation. In particular, no teratogenic effect of bentazone was evident.

Conclusion

Before the re-evaluation of the study with historical control data, the **NOAEL for systemic parental toxicity** was **800 ppm (defined as about 56 mg/kg bw/day considering substance intake within the gestation phase)** based on a reduced body weight in female rats of the F0 and F1 generations and in the F1 males at the highest dose level. A re-evaluation of the study, including historical control data, a check for maternal toxicity and litter size effect, a parental NOAEL is now proposed of 200 ppm based on the reduced feed consumption in the F0 females between PND 1 and 4. The substance intake during lactation period PND1-4 is used as the maternal feed intake was mainly reduced during this period. This is equivalent to 22 mg/kg bw/day (mean substance intake of F0-dams between PND1-4 during lactation)

The **NOAEL for the offspring was 200 ppm (about 14 mg/kg bw/day considering substance intake during the gestation phase) and is after re-evaluation, including historical data proposed as 22 mg/kg bw/day considering the more relevant time frame of PND1-4** since a reduced body weight of the F1 and F2 pups was observed in the two upper dose groups secondary to maternal toxicity. The substance intake

during lactation period PND1-4 is proposed instead of the intake during gestation as the fetal body weight was not reduced at birth but from PND4. No substance-induced gross-pathological or histopathological changes occurred.
No teratogenic effects were detected. The fertility, rearing behavior and development of the pups was comparable in all groups. The **reproductive NOAEL** is set at 164.3 mg/kg bw.

3.10.1.3 Study 3 – developmental study, rat

The main study was preceded by a dose-finding study which is briefly described under this section, too.

Anonymous 1991 (Agrichem file no. R 463)
Dose range-finding embryotoxicity study (including teratogenicity) with bentazon in the rat; Agrichem file no. R 463
Testing facility: date of experimental work completed: 25 April 1990
Previous evaluation:in original DAR

Test procedure: 20 female Wistar rats (strain: WIST Hanlbm: Wist (SPF), source: Biological Research Lab., Fuellinsdorf, Switzerland) were mated and allocated to the control and three test groups consisting of five animals each. A formulation containing 600 g bentazone sodium salt/1 (batch no. B016) was administered from day 6 through day 15 post coitum (p.c.) once daily by oral gavage at dose levels of 0 (vehicle control); 50; 150 and 450 mg/kg bw/day in bi-destilled water. On day 21 p.c., the dams were killed and subjected to gross examination with emphasis upon the uterus. Fetuses were developed by caesarian section and grossly examined.
Results: Mortality or clinical signs did not occur in females throughout the study. Food consumption tended to be slightly diminished at the top dose level but statistical significance was not reached. The slightly reduced body weight after the treatment period at the two upper dose levels was caused by the lower number of fetuses in these groups and did not reflect a true systemic effect on the females. At necropsy, no abnormal findings were noted in any of the dams. There was a dose-dependent increase in post-implantation losses in all treated groups reaching statistical significance at the two upper dose levels (9.7% in control group versus 13.6%, 29.3% and 39.0% in low, mid and high dose group). The corresponding total number of fetuses and of fetuses per dam were decreased in the groups receiving 150 and 450 mg/kg bw/day. The occurrence of fetal resorptions and a reduced mean fetal weight were confined to the highest dose level (6.4% decrease on individual basis, and 8.5% decrease on a litter basis). However, dead or abnormal fetuses were not noted in any treated group.
Conclusion: Dosages of 5, 30 and 180 mg/kg bw/day were chosen for the main developmental toxicity study in the rat.

Main study

Anonymous 1991 (Agrichem file no. R 22)

Embryotoxicity study (including teratogenicity) with bentazon in the rat; Agrichem file no. R 22

Testing facility: date of experimental work completed: 18 October 1990

Previous evaluation:in original DAR

Material and methods

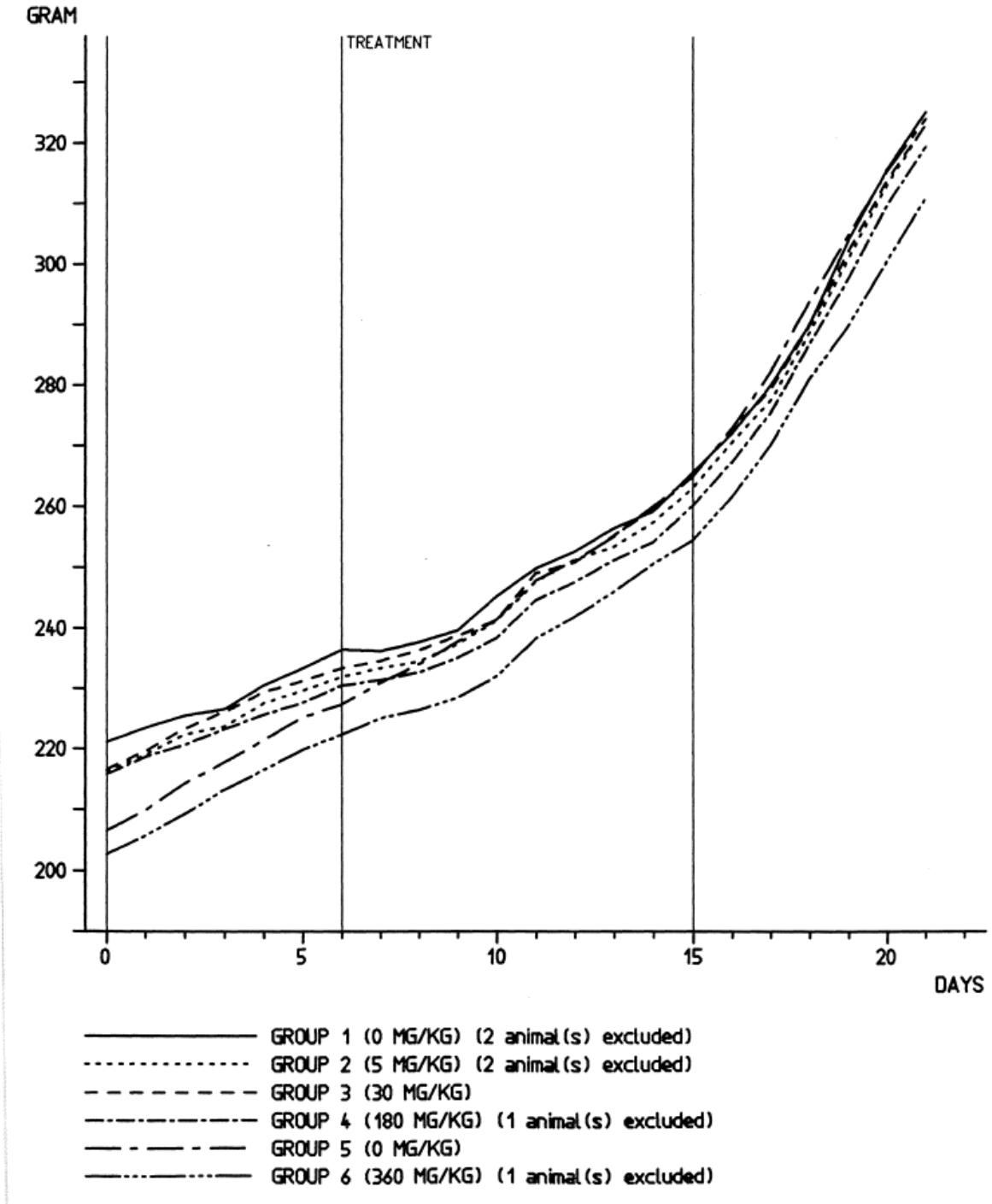
Test method: The study was performed according to OECD guideline 414 and EPA/FIFRA, Subdivision F, guideline 83-3 and is in compliance with the demands of Directive 87/302/EEC, May 30, 1987, therefore.

Deviations: None.

GLP: Yes. When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP. A formal GLP Compliance and a Quality Assurance Statement are included in the report.

Acceptance: The study is considered acceptable.

Test system: 25 mated Wistar rats (strain: WIST Hanlbm: Wist (SPF), source: Biological Research Lab., Fuellinsdorf, Switzerland) per group were administered the test article (a formulation of 600 g bentazone sodium salt/L; batch no. B016) dissolved in bi-destilled water from day 6 - 15 p.c. by oral gavage. The dose levels were 0 (vehicle control); 5; 30 and 180 mg/kg bw/day. Due to the results mentioned below, the study was extended by a supplementary dose group of 25 females receiving 360 mg/kg bw/day and a second corresponding vehicle control group of the same size. The rats were housed singly under controlled conditions and received standardized diet and tap water ad libitum. Fresh test substance preparations were made up daily prior to administration. Clinical signs and mortality were checked twice daily. Body weight was determined daily from day 0 until day 21 post coitum (p.c.). Food consumption was recorded on days 6, 11, 16 and 21 p.c. On day 21 p.c., all females were sacrificed and the fetuses removed by caesarean section. Postmortem examinations including macroscopic examination of all internal organs with emphasis upon uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea were performed. The fetuses were sexed, weighed and examined for gross external abnormalities.



Statistics: Univariate one-way analysis of variance, Dunnett test, Steel test and Fisher's Exact test were applied.

Results

Analysis: The test substance/vehicle mixtures were stable over two hours. The mean actual concentrations were in the range from 97.8% to 100.7% of the nominal dose levels. The homogeneity of the substance/vehicle mixtures was also confirmed.

General observations: No mortalities and no clinical signs of reaction to treatment were observed in any of the dose groups. Up to the dose level of 180 mg/kg bw/day, food consumption and body weight in the groups receiving the test article were similar to the control group. At 360 mg/kg bw/day, food consumption was reduced by 8.8% during the first half of the treatment period. The body weight was significantly lower as compared to the control group from day 8 until day 21 p.c. Necropsy of the dams did not reveal any abnormal findings.

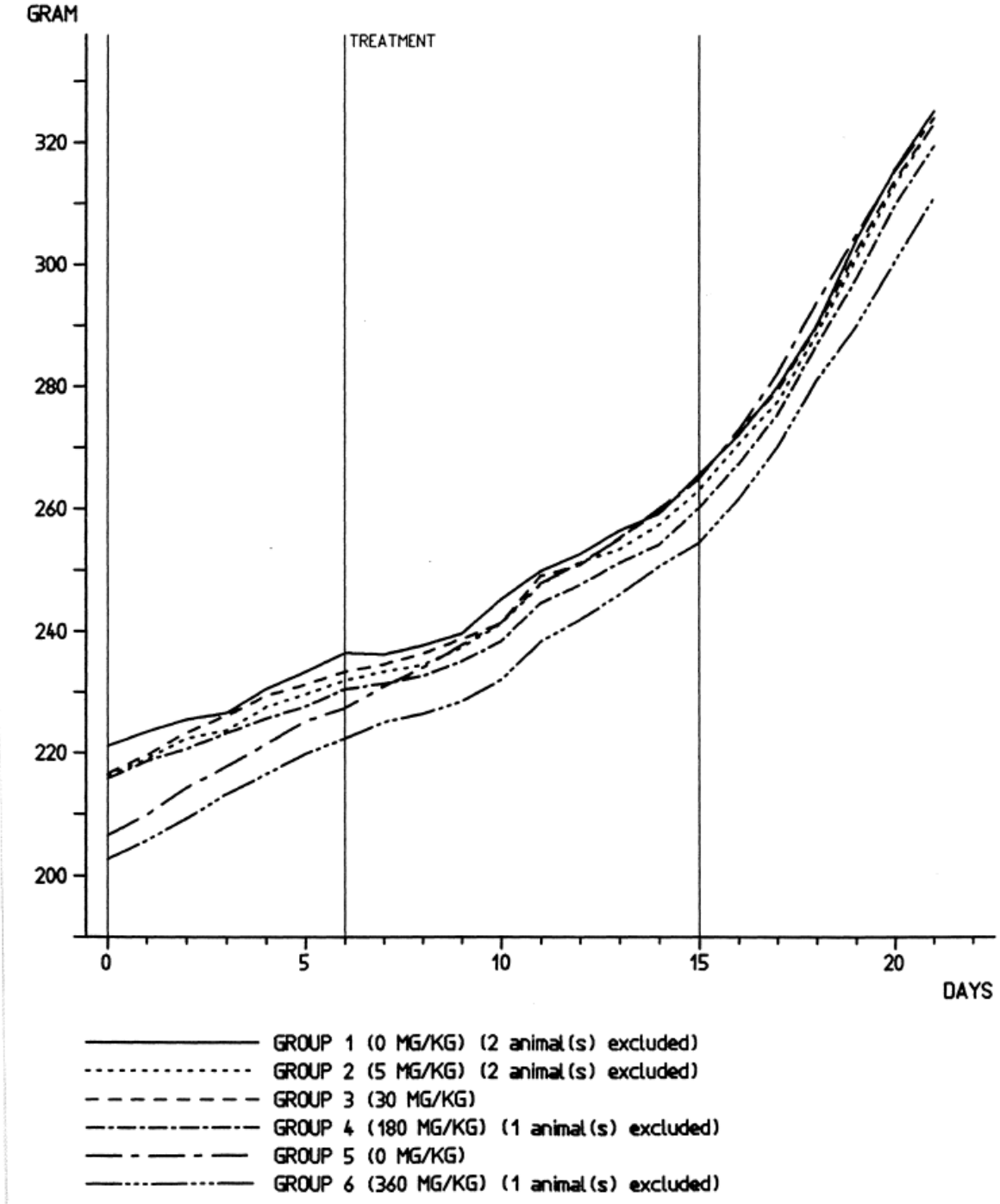


Table 3.10.1.3-1: maternal body weight.

Group	**1**	**2**	**3**	**4**	**5**	**6**
Dose (mg/kg bw)	**0**	**5**	**30**	**180**	**0**	**360**
Day 0	221	216	217	216	207	203
Day 1	223	219	220	219	210	206
Day 2	225	222	223	221	214	209
Day 3	227	224	226	223	218	213
Day 4	231	228	229	226	221	217
Day 5	223	230	231	228	225	220
Day 6	236	232	233	230	227	222
Day 7	236	233	235	231	231	225
Day 8	238	234	236	233	234	**226* (-3.4%)**
Day 9	240	237	239	235	238	**228** (-4.2%)**
Day 10	245	241	241	238	241	**232** (-3.7%)**
Day 11	250	248	249	245	248	**238** (-4.0%)**
Day 12	253	251	251	248	251	**242* (-3.6%)**
Day 13	256	253	255	251	255	**246* (-3.5%)**
Day 14	259	257	260	254	260	**251** (-3.5%)**
Day 15	266	263	265	260	265	**255** (-3.8%)**
Day 16	272	271	273	267	273	**262** (-4.0%)**
Day 17	280	278	279	275	282	**270** (-4.3%)**
Day 18	290	289	290	287	294	**281** (-4.4%)**
Day 19	304	301	302	298	305	**290** (-4.9%)**
Day 20	316	313	314	310	316	**301* (-4.7%)**
Day 21	325	323	323	320	324	**311* (-4.0%)**

Reproduction and fetal parameters: Reproduction parameters were not influenced by treatment. There was no increase in the number of malformed fetuses or fetuses with variations in any dose group. The only treatment-related effect observed was a slightly reduced mean fetal weight at the highest dose level of 360 mg/kg bw/day.

Table 3.10.1.3-2: fetal body weight

Group	**1**	**2**	**3**	**4**	**5**	**6**
Dose (mg/kg bw)	**0**	**5**	**30**	**180**	**0**	**360**
Fetal body weight (litter basis)						
- Total	4.8	4.9	4.9	5.0	4.8	4.7
- Males	4.9	5.0	5.0	5.1	4.9	4.8
- Females	4.7	4.8	4.8	4.8	4.7	**4.5* (-4.3%)**
Fetal body weight (individual basis)						
- Total	4.8	4.9	4.9	4.9	4.8	4.6
- Males	4.9	5.0	4.9	5.1	4.9	4.7
- Females	4.7	4.8	4.8	4.8	4.7	4.5

Conclusion

The slight effects on food consumption and body weight in dams and pups are not considered adverse. **The NOAEL for both maternal and developmental toxicity are set at 360 mg/kg bw/day.** No evidence of teratogenicity was found.

3.10.1.4 Study 4 – developmental toxicity, rat

Anonymous 1986 (Doc. No. 86/421)

Embryotoxicity (including teratogenicity) study with bentazone technical in the rat; BASF Reg. Doc. No. 86/421

Testing facility: RCC, Itingen, Switzerland; date of experimental work completed: 17 September 1986

Previous evaluation:in original DAR

Material and methods

Test method: The study was performed according to OECD guideline 414, EPA/FIFRA, Subdivision F, guideline 83-3 and Japan MAFF guidance 4200 and is in compliance with the demands of Directive 87/302/EEC, May 30, 1987.

Deviations: None.

GLP: Yes. When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP. A formal GLP Compliance and a Quality Assurance Statement are included in the report.

Acceptance: The study is considered acceptable.

Test system: Bentazone (purity: 97.8%, batch no. N 187) was administered as 4% aqueous CMC solution to pregnant female Wistar rats (source: Madoerin AG, Fuellinsdorf, Switzerland) from day 6-15 p.c. by oral gavage. Dose levels were 0 (vehicle control); 40; 100 and 250 mg/kg bw/day. Each dose group consisted of 25 rats. The animals were housed singly under controlled conditions and received standardized diet and tap water ad libitum. The test substance preparations were made up daily prior to administration. Clinical observations and mortality were checked twice daily. Body weight was determined daily from day 0 until day 21 p.c. Feed consumption was recorded on days 6, 11, 16 and 21 p.c. On day 21 p.c., all females were sacrificed and the fetuses removed by caesarean section. Postmortem examinations including macroscopic examination of all internal organs with emphasis upon uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea were performed. The fetuses were sexed, weighed and examined for gross external abnormalities

Statistics: Dunnett test for body weight and food consumption; for reproduction data Fisher's Exact test.

Results

Analysis: The test substance/vehicle mixtures were stable over two hours. The analysis of the mean actual concentrations confirmed the intended dose levels (40 mg/kg bw/day: 101.1% of theoretical value; 100 mg/kg bw/day: 106.8% of theoretical value; 250 mg/kg bw/day: 92.3% of theoretical value). The homogeneity of the substance/vehicle mixtures was also confirmed.

General observations: The treatment of mated female rats with bentazone did not show any clinical signs. However, a statistically significant reduction of the mean food consumption in the highest dose group

receiving 250 mg/kg bw/day which became apparent between days 6 and 11 was considered test substance-related. However as the effect was only sligh (-5.6%) the effect was not considered to be adverse.

Table 3.10.1.4-1: Food consumption and body weight development in rat administered Bentazone during days 6 to 15 of gestation

Dose level [mg/kg]	0	40	100	250
Food consumption [g/animal/day]				
Day 0 to 6	19.8	19.9	20.2	19.9
Δ%		+0.5	+2.0	+0.5
Day 6 to 11	21.3	21.1	20.6	**20.1***
Δ%		-0.9	-3.3	**-5.6**
Day 11 to 16	22.5	21.9	22.1	21.8
Δ%		-2.7	-1.8	-3.1
Day 16 to 21	23.0	22.3	22.5	22.1
Δ%		-3.0	-2.2	-3.9
Body weight gain [g]				
Day 0 to 6	22	21	25	23
Δ%	10.9	10.4	12.8	11.4
Day 6 to 11	19	18	15	16
Δ%	8.5	8.1	6.8	7.1
Day 11 to 16	26	23	26	25
Δ%	10.7	9.6	11.0	10.4
Day 16 to 21	51	49	51	43
Δ%	19	18.6	19.5	16.2
Day 6 to 21	96	90	92	84
Δ%	43.0	40.5	41.6	37.5

* $p < 0.05$,

Reproduction and fetal parameters: The investigation of reproduction data revealed a test substance-related, statistically significant increase of the post-implantation loss (number of fetal resorptions increased to 14.4%) in the dams of the 250 mg/kg bw/day group, with correspondingly reduced number of living fetuses. In addition, mean fetal body weight was reduced at the top dose group (250 mg/kg bw/day) by 10.4% in comparison to that of the vehicle control group.

Table 3.10.1.4-2: Total number of fetal resorptions, mean number of living fetuses per litter and mean fetal weight

Dose level (mg/kg bw/d)	**0**	**40**	**100**	**250**
Fetal resorptions (total)	0	0	1	44
Mean number of living fetuses per litter	10.9	10.6	11.4	9.5
Mean fetal weight (g)	4.8	4.9	4.9	4.2

All other reproduction parameters remained uninfluenced. During the investigations of fetuses (external, gross-pathological and skeletal investigations), no abnormal findings were noted except incompletely ossified skeletons in the 250 mg/kg bw/day group fetuses. This isolated finding was assessed as a consequence of a delayed maturation as indicated by the decreased fetal body weight.

Conclusion

The **NOAEL for maternal toxicity** was **250 mg/kg bw/day,** the highest dose tested. Developmental toxicity was confined to the top dose group and was characterized by an increased fetal resorption rate, a decreased fetal body weight and incomplete ossification. Thus, the **developmental NOAEL** was **100 mg/kg bw/day.** Bentazone did not show any teratogenic effects up to the highest dose level of 250 mg/kg bw/day under the described conditions of this study.

3.10.1.5 Study 5 – developmental toxicity, rat

Anonymous 1982 (Doc. No. 84/066)

Teratogenicity study of bentazone, Reg. No. 51 929 (ZNT No. 81/273) in rats by dietary administration; BASF Reg. Doc. No. 84/066

Testing facility: ; date of experimental work completed: 19 November 1981

Previous evaluation:in original DAR

Material and methods

Test method: No specific test guideline was mentioned in the report. However, the study procedure to a great extent was in compliance with the demands of Directive 87/302/EEC, May 30, 1987.

Deviations: Dietary administration was the route chosen. Duration of the administration period was from day 0 to day 21 and, thus, exceeded the period of major organogenesis.

GLP: Yes. When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP. A formal GLP Compliance and a Quality Assuarance Statement are included in the report.

Acceptance: The study is considered supplementary only since the route of administration and duration of treatment does not allow a comparison with other studies on teratogenicity. However, it provides important additional information.

Test system: Bentazone (purity: 93.9%, batch no. N 169) was administered to groups of 23 female SD/CRJ rats (source: Charles River Japan Co., Ltd.) at dose levels of 0; 2000; 4000 or 8000 ppm as admixture in the diet. Treatment commenced on day 0 of gestation and was finished on day 21 when the dams were sacrificed and necropsied. One female out of 23 in the control and two animals in the low dose group did not become pregnant and were therefore excluded from investigation. Male animals were housed singly, female animals

were housed in groups of five each until copulation and singly after copulation under controlled conditions. All animals had free access to standardized diet and tap water. The test substance was added to the diet at one time before starting the treatment period. Clinical observations were made up twice daily. Body weight was determined on day 0 of pregnancy and later on together with food and water consumption daily from day 1 to 21 of pregnancy. The fetuses were dissected from the uterus, weighed, sexed and checked for any morphological abnormalities (external, gross-pathological and skeletal examinations).
Statistics: Student's t-test

Results

Analysis: The analysis of the dietary concentrations confirmed the intended dose levels (2000 ppm: 77.8 - 119.9% of theoretical value; 4000 ppm: 85.4 - 110.2% of theoretical value; 8000 ppm: 93.0 - 106.7% of theoretical value) .
Substance intake: The mean daily substance intake was 162 mg/kg bw/day in the 2000 ppm group; 324 mg/kg bw/day in the 4000 ppm group and 631 mg/kg bw/day in the 8000 ppm group.
General observations: Clinical symptoms noted were alopecia in one animal of the 8000 ppm group, another four animals of this dose group exhibited hematuria and brownish urine, depression, skin pallor, piloerection and nasal hemorrhage from day 19 of gestation onwards. No deaths occurred in either the treated groups or the control group. Body weight gain in the highest dose group was reduced (-6.5% at day 21). Food consumption was increased in the 2000 ppm (11.5% at day 1) and 4000 ppm (12.5% at day 21) groups, whereas a significant decreases was noted in the 8000 ppm group (-37.5% at day 21). Water consumption in the 4000 ppm (11.6%) and 8000 ppm (18%) groups was significantly higher than in the control group. Amniotic fluid weight was increased in groups receiving 4000 ppm (33%) or 8000 ppm (32%). Necropsy findings in animals sacrificed on day 21 of gestation revealed an uterine hemorrhage in one animal of the 8000 ppm group. No abnormalities were noted in all other animals.
Fetal findings: There were no significant differences between implantations, embryos and fetal mortalities in the 2000; 4000 and 8000 ppm groups and those in the control group. In the 8000 ppm group, there was an increased incidence of fetuses with low body weight and some fetuses displayed petechiae in the liver. In addition, ossification of cervical vertebrae was reduced at this top dose level.

Conclusion

A dietary level of 8000 ppm (equivalent to a calculated intake of 631 mg/kg bw/day) exerted marked toxic effects on pregnant animals and their fetuses appeared to suffer from secondary developmental disturbances.
At the mid dose level, water consumption and amniotic fluid weight of the dams were still higher. Thus, the **NOEL for maternal toxicity was 2000 ppm (about 162 mg/kg bw/day)** and the **NOEL for embryo-/fetotoxicity was 4000 ppm (324 mg/kg bw/day)** in this dietary study. No evidence of teratogenicity was observed.

3.10.1.6 Study 6 – developmental toxicity, rat

Anonymous 1971 (Doc. No. 71/0041)

Bericht über die Prüfung von 3-Isopropyl-2,1, 3-benzo-thiadiazinon- (4)-2,2-dioxide (= Bentazone) auf etwaige teratogene Wirkung an der Ratte bei peroraler Applikation; BASF Reg. Doc. No. 71/0041

Testing facility: date of test report: 29 July 1971

Previous evaluation:in original DAR

Material and methods

Test method: The study was run according to the "Guidelines for reproduction studies for safety evaluation of drugs for human use" (FDA, 1966). The study procedure is not in compliance with the demands of Directive 87/302/EEC, May 30, 1987.

GLP: No. This study was performed prior to implementation of GLP guidelines.

Acceptance: The study is considered supplementary only since it does not comply with current standards. The outcome of this study is rather equivocal (see conclusion).

Test system: Bentazone technical (batch no. not available) was administered as 1.0% aqueous tylose suspension to pregnant female Sprague-Dawley rats (source: Gassner Co., Ottobrunn, Germany) on days 6 - 15 of gestation by oral gavage. Dose levels were 0; 22.2; 66.7 and 200 mg/kg bw/day. Treated groups consisted of 20-32 animals. Two control groups were included (see Table 3.10.1.6-1). The rats were housed in pairs under controlled conditions and received standardized diet and water ad libitum. The test substance suspensions were made up fresh every day. Clinical observations were recorded daily. Body weight was determined three times a week and on day 20 p.c. On this day, all animals were sacrificed and necropsied. The fetuses were dissected from the uterus, weighed, sexed and checked for any morphological abnormalities (external, gross-pathological and skeletal examinations).

Statistics: Statistical analysis was not performed.

Results

Analysis: Not performed.

Findings: Following administration of the two lower doses neither maternal toxicity nor embryo-/fetotoxic effects could be detected. In the highest dose group receiving 200 mg/kg bw/d, the resorption rate was drastically increased. In addition, the fetuses showed a decrease in body weight, an increase in the number of runts (13.4%) and an increase in the frequency of anasarca (11.5%). The occurence of anasarca was confined to this group.

Table 3.10.1.6-1: Resorption rate and number of runts

Dose level (mg/kg bw/d)	**0 (un-treated control)**	**0 (vehicle control)**	**22.2**	**66.7**	**200**
Pregnant dams	36	32	20	24	32
Resorptions (total)	32	25	13	25	256
Resorption rate (% of all implantations)	7.6	6.5	5.5	8.2	66.3
Living fetuses	384	359	212	279	127
Number of runts (total and percentage)	3 (0.6)	3 (0.8)	4 (1.9)	2 (0.7)	17 (13.4)

The total summary incidence of fetuses with anomalies of all types was also elevated. In contrast, maternal toxicity was not observed at this dose level.

Conclusion

The NOEL was 200 mg/kg bw/day for maternal toxicity and 66.7 mg/kg bw/day for embryo-/fetotoxicity. The high resorption rate and the fetal findings at the top dose level might suggest a fetotoxic or teratogenic potential of the test compound. However, it is noteworthy to mention that the embryo-/fetotoxic effects were not reproducible when the study was repeated six years later on the same species and strain and using the same dosages of the test substance (see section 3.10.1.7 below).

3.10.1.7 Study 7 – developmental toxicity, rat

Anonymous 1978 (Doc. No. 78/039)

Investigation to determine the prenatal toxicity of 3-isopropyl-2,1,3-benzothiadiazin-4-one-2,2-dioxide on rats; BASF Reg. Doc. No. 78/039

Testing facility: date of experimental work completed: 12/1977

Previous evaluation:in original DAR

Material and methods

Test method: The test procedure was in accordance with the "Guidelines for reproduction studies for safety evaluation of drugs for human use" (FDA, 1966) and the "Guidance on reproduction studies from The Association of British Pharmaceutical Industry" (1975), respectively. The study was not in compliance with the demands of Directive 87/302/EEC, May 30, 1987.

GLP: No. The study was performed prior to implementation of GLP guidelines.

Acceptance: The study is considered supplementary only since it does not comply with current standards.

Test system: Bentazone (purity: 92.5%, batch no. not available) was administered by oral gavage as 1% aqueous CMC suspension to female Sprague-Dawley rats (source: Wiga Co., Sulzfeld, Germany) on days 6 - 15 of gestation. Dose levels were 0; 22.2; 66.7 and 200 mg/kg bw/day. Each group consisted of 26 - 29 rats. The animals were housed 2 per cage under controlled conditions and had free access to standardized diet and drinking water. Clinical observations and mortality were checked daily. Body weight was determined three times a week, on day 0 of pregnancy and on day 6, 11, 15 and 2 0 p.c. On day 20, all animals were sacrificed and necropsied. The fetuses were dissected from the uterus, weighed, sexed and checked for any morphological abnormalities (external, gross-pathological and skeletal examinations)
Statistics: Williams t-test, Fisher's exact test and Mann-Whitney-U-test.

Results

Analysis: Not performed.
Findings: Bentazone was tolerated by all animals without any clinical symptoms and with no adverse effect on body weight and body weight gain. No animal died during the study period. No gross-pathological changes were found. No difference between control group and substance-treated groups were noted with respect to conception rate, number of live or dead implantations or resorptions. Body weight of fetuses, their length and placenta weight remained unaffected. The examination of the fetuses did not reveal any abnormal findings.

Conclusion

Under the conditions of this study, no embryo-/fetotoxic or teratogenic effects were noted. The **NOEL for both maternal and embryo-/fetotoxicity was 200 mg/kg bw/day.** The evidence of a fetotoxic or tetatogenic potential of bentazone obtained in a previous study (see section 3.10.1.6) was not confirmed.

3.10.1.8 Study 8 - Published literature: developmental toxicity, rat

El-Mahdi MM and Lofti MM (1988)

Teratological effects of pesticides (Basagran) on embryo of albino rat; BASF Reg. Doc. No. 88/10538
Testing facility: Cairo University, Giza, Egypt
Previous evaluation:in original DAR

Groups of three pregnant albino rats (strain and source not specified) were orally administered single doses of 0; 25; 90 or 200 mg of the formulation Basagran (origin and purity not submitted)/kg bw (corresponding to 0; 12.0; 43.2 or 96 mg of bentazon/kg bw) by gavage on the sixth, eighth, 11th, 14th or 16th day of gestation. On day 20 p.c. all animals were sacrificed and necropsied. The fetuses were dissected from the uterus. Resorptions were counted and the skeletons of fetuses were examined. The fetal findings observed

consisted of an increased resorption rate, retardation of fetal development, incomplete ossification and absence of some bones. The increased resorption rates were noted at comparable incidences in all treated groups, irrespective of the dose administered. The incidence and severity of the findings decreased with the later times of administration. Thus, the findings were time-dependent and not dose-dependent. The publication gives no details on maternal toxicity. The results of the gross-pathological examination of the fetuses were only summarized in this study and the frequency of the changes was not reported. In addition, the results of the examination of the control animals were not given. Due to the inconsistency of the data reported, this investigation is considered unacceptable for evaluation purposes. However, it provides supplementary information since time-dependence of fetal effects was investigated.

3.10.1.9 Study 9 – developmental study, rabbit

Anonymous 1984 (Doc. No. 84/048)

Study to determine the prenatal toxicity of 3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4 (3H)-on-2,2-dioxide in rabbits; BASF Reg. Doc. No. 84/048

Testing facility: date of experimental work completed: 09/1977 (date of original german report 6 March 1978)

Previous evaluation:in original DAR

Material and methods

Test method: Investigations were carried in accordance with the "Guidelines for reproduction studies for safety evaluation of drugs for human use" (FDA, 1966) and the "Guidance on reproduction studies from The Association of the British Pharmaceutical Industry" (1975). The study procedure is not in compliance with the demands of Directive 87/302/EEC, May 30, 1987.

GLP: No. This study was performed prior to implementation of GLP guidelines.

Acceptance: The study considered supplementary only since it does not comply with current guidelines.

Test system: Bentazone (purity 92.5%, batch no. not available) was administered as 0.5% aqueous CMC solution by oral gavage to groups of 15 female Himalayan ChBB:HM rabbits (source: Dr. Thomae GmbH, Biberach, Germany) per dose group on days 6 - 18 of gestation. Does had been artificially inseminated. Dose levels were 0; 50; 100 and 150 mg/kg bw/day. The animals were housed singly under controlled conditions and received a daily ration of 130 g of standardized diet. Drinking water was available ad libitum. Clinical observations and mortality were checked daily. Feed consumption was recorded daily. Body weight was determined three times a week, on the day of insemination and on day 6, 12, 18 and 2 8 post insemination (p.i.). On day 28 p.i., all females were sacrificed and subjected to gross-pathological examination. The fetuses were dissected from the uterus, weighed and checked for any morphological abnormalities (external, internal and skeletal examinations).

Statistics: Williams t-test.

Results

Analysis: Not performed.

Findings: Neither clinical signs of toxicity nor any adverse effect on body weight and body weight gain were noted at any dose level. One animal in each of the 100 mg/kg bw and 150 mg/kg bw groups died. However, since no pathological changes of the internal organs were detected macroscopically, this death is not likely to be test substance-related. Similarly, no gross-pathological changes were recorded in any other doe. However, despite the unchanged body weight, a substantial loss of adipose tissue was apparent in the females receiving the mid and the high doses. The conception rate, the mean number of live and dead implantations, the body weight of the fetuses, their length and the placenta weights remained unaffected by the treatment. Type and number of the skeletal findings, which were classified as anomalies, variations and/or retardations, recorded for the 50; 100 and 150 mg/kg bw fetuses were substantially similar to actual control values. Thus, no teratogenic effects were found.

Table 3.10.1.9-1: Incidence of skeletal malformations and variations

Dose level [mg/kg]	**0***	**0****	**50**	**100**	**150**
Litters Evaluated	14	15	14	11	13
Fetuses Evaluated	82	83	61	58	59
Live	82	83	61	58	59
Dead	1	0	0	0	0
Total skeletal abnormal findings					
- Fetal incidence	65	58	45	41	39
- Litter incidence	14	13	14	10	12
Individual skeletal abnormal findings,					
Skull: retinal fold unilaterally	2	3			
Ribs: accessory rib bilaterally					2
Ribs: accessory rib unilaterally		3	1		
Sternum: aplasia of individual sternebrae	32	23	19	15	9
Sternum: fused sternebrae	1				
Sternum: partial ossification of individual sternebrae	31	31	23	26	30
Sternum: dislocation of individual sternebrae		1	2		2

* control group untreated
** control group treated with CMC

Conclusion

Under the conditions of this study, the **NOAEL for both maternal toxicity and embryo-/fetotoxicity was 150 mg/kg bw.** No evidence of teratogenicity was observed.

3.10.1.10 Study 10 – developmental toxicity, rabbit

Anonymous 1987 (Doc. No. 87/058)

Embryotoxicity (including teratogenicity) study with bentazon technical in the rabbit; BASF Reg. Doc. No. 87/058

Testing facility: date of experimental work completed: 28 October 1986

Previous evaluation:in original DAR

Material and methods

Test method: The study was performed according to OECD guideline 414; EPA/FIFRA, Subdivision F, guideline 83-3 and Japan MAFF guidance no. 4200 and is in compliance with the demands of Directive 87/302/EEC, May 30, 1987.

Deviations: None.

GLP: Yes. When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP. A formal GLP Compliance and Quality Assurance Statement are included in the report.

Acceptance: The study is considered acceptable.

Test system: 16 pregnant Chinchilla rabbits (source: Madoerin AG, Fuellinsdorf, Switzerland) per group were adminstered bentazone (purity: 97.8%, batch no. N 187) as 4% aqueous CMC suspension by oral gavage on days 6 - 18 p.c. Dose levels were 0; 75; 150 and 375 mg/kg bw/day with the control group receiving the vehicle only. These dosages were based upon the results of a preliminary dose-finding study. The animals were housed singly under controlled conditions and received standardized diet and tap water ad libitum. The test substance preparations were made up daily prior to administration. Clinical observations and mortality were checked twice daily. Body weight was determined daily from day 0 to day 28 p.c. Feed consumption was recorded on day 6, 11, 15, 19, 24 and 28 p.c. On gestation day 28, the does were sacrificed and subjected to gross pathology including assessment of reproduction parameters like number of corpora lutea, implantations and resorptions. The fetuses were dissected from the uterus, weighed and checked for any morphological abnormalities (external, gross-pathological and skeletal examinations).

Statistics: Dunnett test for body weight and food consumption; Kruskal Wallis test and Fisher's Exact test for reproduction parameters

Results

Analysis: The test substance/vehicle mixtures were stable over two hours. The analysis of the actual mean concentrations confirmed the intended dose levels (75 mg/kg bw/day: 91.7 - 101.2% of theoretical value; 150 mg/kg bw/day: 85.1 - 103.1% of theoretical value; 375 mg/kg bw/day: 85.7 - 100.3% of theoretical value). The homogeneity of the substance/vehicle mixtures was also confirmed.

Findings: A reduction of the mean food consumption was noted in the 375 mg/kg bw/day group during the treatment period. In one dam of the highest dose group (375 mg/kg bw/day), five aborted placentae were found on day 22 p.c. In this dam, a total post-implantation loss was ascertained during necropsy on day 28 p.c. One more high dose doe was found non-pregnant. No test substance-related differences in comparison to the vehicle control group data were noted in the remaining parameters recorded for the top dose animals and for all data of the 75 mg/kg bw/day and 150 mg/kg bw/day groups. During gross-pathological investigations, a single incidental finding (hydrocephalus internus) in one fetus of the 150 mg/kg bw/day group was noted. During the skeletal investigations, isolated findings were noted in all groups, including the control. There were no signs of test substance-relationship. No substance-induced teratogenicity was observed.

Table 3.10.1.10-1: Food consumption and body weight development in rabbits administered Bentazone during days 6 to 18 of gestation

Dose level [mg/kg bw/day]	0	75	150	375
Food consumption [g/animal/day]				
Day 0 to 6 Δ%	191	204 + 6.8	186 -2.6	197 +3.1
Day 6 to 11 Δ%	189	209 +10.6	194 +2.6	186 -1.6
Day 11 to 15 Δ%	195	197 +1.0	185 -5.1	175 -10.3
Day 15 to 19 Δ%	202	204 +1.0	216 +4.8	186 -7.9
Day 19 to 24 Δ%	177	165 -6.8	171 -3.4	169 -4.5
Day 24 to 28 Δ%	165	134 -18.8	158 -4.2	157 -4.8
Day 6 to 19 Δ%	**195**	**204 +4.6**	**198 +1.5**	**183 -6.2**
Body weight [g]				
Day 0 Δ%	2869	2748 -4.2	2799 -2.4	2823 -1.6
Day 6 Δ%	3062	2947 -4.8	3002 -2.0	2970 -3.0
Day 11 Δ%	3127	3045 -2.6	3050 -2.5	3018 -3.5
Day 15 Δ%	3196	3124 -2.3	3106 -2.8	3078 -3.7
Day 19 Δ%	3260	3197 -1.9	3190 -2.1	3134 -3.9
Day 24 Δ%	3351	3259 -2.7	3262 -2.7	3229 -3.6
Day 28 Δ%	3433	3336 -2.8	3355 -2.3	3324 -3.2
Body weight gain [g]				
Day 0 to 6 Δ%	193	199 +3.1	203 +5.2	147 -23.8
Day 6 to 11 Δ%	65	98 +50.8	48 -26.2	48 -26.2
Day 11 to 15 Δ%	69	79 +14.5	56 -18.8	60 -13.0
Day 15 to 19	64	73	84	56

Table 3.10.1.10-1: Food consumption and body weight development in rabbits administered Bentazone during days 6 to 18 of gestation

Dose level [mg/kg bw/day]	0	75	150	375
Δ%		+14.1	+31.3	-12.5
Day 19 to 24 Δ%	91	62 -31.9	72 -20.9	95 +4.4
Day 24 to 28 Δ%	82	77 -6.1	93 +13.4	95 +15.9
Day 6 to 19 **Δ%**	**198**	**250** **+26.3**	**188** **-5.1**	**164** **-17.2**
Day 6 to 28 Δ%	371	389 +4.9	353 -4.9	354 -4.6
Corrected body weight gain				
In %*	-1.5	-0.8	-3.2	-2.8

* based on bodyweight day 6

Table 3.10.1.10-2: Mean relative corrected body weight change of pregnant rats administered Bentazone during Days 6 to 18 of gestation

Dose level [mg/kg bw/day]	**0**	**75**	**150**	**375**
Gravid uterus (g)[+]	420	412	452	444
Carcass (g) [+] (terminal body weight minus uterine weight)	3013	2924	2903	2880
Net weight change from Day 6 (g) [+] (carcass weight minus Day 6 p.i. body weight)	-49	-23	-99	-90
Corrected body weight gain In % §	-1.5± 3.4	-0.8 ± 3.4	-3.2 ± 3.3	-2.8 ± 4.3

Statistical evaluation: * $p \leq 0.05$

[+]: calculated by the author based on the individual values given in the report

§ Calculated as (Weight on day 28) -(Weight on day 6) -(Uterus weight) and expressed as weight gain in percent of weight on day 6 (Mean ± SD).

Table 3.10.1.10-3: Caesarean section data

Dose level [mg/kg bw/day]	**0**	**75**	**150**	**375**
Pregnancy status				
- mated [n]	16	16	16	16
- pregnant [n]	16	16	16	15
conception rate [%]	100	100	100	94
- aborted/resorbed [n]	0	0	0	1
- dams with viable fetuses [n]	16	16	16	14
- mortality	0	0	0	0
- pregnant terminal sacrifice [n]	16	16	16	14
Caesarean section data				
- Corpora lutea [mean/dam]	7.8±1.6	7.7±1.5	8.6±1.1	8.9±1.5
total number [n]	125	123	137	124
- Implantation sites [mean/dam]	7.7 ±1.9	7.4±1.5	8.4±1.1	8.4±1.9
total number [n]	123	119	134	118
- Pre-implantation loss [%]	1.6	3.3	2.2	4.8
- Post-implantation loss [%]	4.1	4.2	3.7	3.4 (8.8[+])

Table 3.10.1.10-3: Caesarean section data

Dose level [mg/kg bw/day]	**0**	**75**	**150**	**375**
- Resorptions [mean/dam]	0.3	0.3	0.3	0.3
total number [n]	5	5	5	4
% of implantations	4.1	4.2	3.7	3.4
- Early resorptions [mean/dam]	0.3	0.1	0.3	0.1
total number [n]	4	2	4	1
% of implantations	3.3	1.7	3.0	0.8
- Late resorptions [mean/dam]	0.1	0.2	0.1	0.2
total number [n]	1	3	1	3
% of implantations	0.8	2.5	0.7	2.5
- Dead fetuses [n]	0	0	0	0
- Live fetuses [mean/dam]	7.4 ±2.2	7.1±1.6	8.1±1.5	8.1±1.9
total number [n]	118	114	129	114
- Total live female fetuses				
total number [n]	56	56	75	56
Mean [%]	47.5	49.1	58.1	49.1
- Total live male fetuses				
total number [n]	62	58	54	58
Mean [%]	52.5	50.9	41.9	50.9
Mean fetal weight				
- males§ [g]	37.9±3.8	38.5±4.6	37.0±2.6	36.0±3.5
- females§ [g]	37.1±4.5	37.6±5.2	35.7±2.3	35.4±2.9
- males & females§ [g]	37.7±3.9	38.0±4.7	36.4±2.1	35.7±2.8
- males & females [g]	36.8±5.0	37.3±5.7	36.1±3.8	35.3±4.5

This table excludes the dams #53 an #64 of group 4;

+Post implantation loss under consideration of dam #64 (125 implantations and 11 losses)

§ = unweighted mean of litter means and variation between litters

Fetal findings:

Table 3.10.1.10-4: Incidence of visceral (soft tissue) malformations and variations

Dose level [mg/kg bw/day]	**0**	**75**	**150**	**375**
Litters Evaluated	16	16	16	14
Fetuses Evaluated	118	114	129	114
Live	118	114	129	114
Dead	0	0	0	0
Hydrocephalus internus				
- Fetal incidence No. (%)	0	0	1 (0.8)	0
- Litter incidence No. (%)	0	0	1 (6.3)	0

Table 3.10.1.10-5: Incidence of skeletal malformations and variations

Dose level [mg/kg bw/day]	**0**	**75**	**150**	**375**
Litters Evaluated	16	16	16	14
Fetuses Evaluated	118	114	129	114
Live	118	114	129	114
Dead	0	0	0	0
Total skeletal abnormal findings				

Table 3.10.1.10-5: Incidence of skeletal malformations and variations

Dose level [mg/kg bw/day]	**0**	**75**	**150**	**375**
- Fetal incidence - Litter incidence	2 2	6 6	3 2	3 3
Individual skeletal abnormal findings,				
- Bipartite sternebrae no 5	1	3	2	1
- Abnormally ossified sternebrae nos 2-5	0	1	0	0
- Abnormally ossified sternebrae nos 2 and 3	0	1	0	0
- Supernumerary sternebrae (betw. 5&6)	0	0	1	0
- Thoracic vert. body no 13 absent, thoracic vertebral arch no. 13 absent (right side), rib no. 13 absent (scoliosis)	1	0	0	0
- Dumbbell-shaped vertebral body no 7	0	0	0	1
- Absence of 2 ribs, (right side), basal fusion of rib no. 8 with rudimentary rib no. 9, caused broadening in the costovertebral region (distal part of rib no. 9 absent);	0	1	0	0
- Distal part of rib no 8 broadened	0	0	0	1

Conclusion

Based on the slightly reduced food consumption at 375 mg/kg bw/day and on equivocal fetotoxic signs in one high dose female, the **NOAEL for effects on the maternal and fetal organism was 150 mg/kg bw/day.** Bentazone did not show any teratogenic effects up to the highest dose level of 375 mg/kg bw/day under the conditions of this study.

3.10.2 Human data

No data

3.10.3 Other data (e.g. studies on mechanism of action)

3.10.3.1 Published literature 1 - Spematogenesis

Garagna et al. 2005

Previous evaluation: submitted for renewal (supplementary)

To determine whether low doses of bentazone affect spermatogenesis, it was dissolved in water at the concentration of 30 µg/L. Bentazone (purity not reported) was administered through drinking water to: (1)

adult CD-1 mice (10 males/dose) for 100 days resulting in a dose of 21 µg/kg bw/d and (2) mice exposed in utero and during lactation (12 males from 3 dams) and for 100 days after birth resulting in a dose of 14 µg/kg bw/d. The results show that there were no effects of bentazone on spermatogenesis. The potential genotoxic effects were evaluated on spermatozoa (Comet assay) in pachytene spermatocytes (analysis of the synaptonemal complex) and in bone marrow cells (frequency of micronuclei). No evidence of a genotoxic effect of bentazone was observed. The only significant effect was that the frequency of stage VII, IX and XII of the cycle of the somniferous epithelium of adult mice and of stages I, III and VII of mice exposed in utero and for 100 days after birth was different when compared to that of control mice.

According to a review article by Creasy 1997 (http://tpx.sagepub.com/content/25/2/119, page 12) quantitating stage frequency is not an appropriate endpoint. The endpoint should only be used to identify cell loss in case of spermatid retention.

3.10.3.2 Published literature 2 – hormonal activity

Orton et al. 2009

Previous evaluation: submitted for renewal (supplementary)

Twelve environmentally relevant pesticides, including bentazone, were tested for their endocrine disrupting potential in two *in vitro* assays. A recombinant yeast screen was used to detect receptor mediated (anti-)estrogenic (YES-assay) and (anti-)androgenic activity (YAS-assay) (concentration range 0.01 µM – 1000 µM) and cultured *Xenopus* oocytes were used to measure effects on the ovulatory response and ovarian steroidogenesis (concentration range 0.000625 – 62.5 µM). Cytotoxicity in the respective tests was evaluated. Bentazone induced anti-androgenic transcriptional activity at 500 – 1000 µM in the YAS assay after co-incubation with 2.5 nM dihydrotestosteron. An androgenic transcriptional activity was not observed. Bentazone showed no effect in the YES-assay. In addition, no effect of bentazone was observed in the ovulation assay.

3.10.3.3 Published literature 3 – hormonal activity

Roncaglioni et al. 2008

Previous evaluation: submitted for renewal (supplementary)

The aim of the study was to provide an insight into the use of QSAR models to address ED effects mediated through the estrogen receptor (ER). Bentazone is listed as part of the training set based on the Japanese METI database. The database is one of the largest collection of data for ER publically available, with more

than 900 compounds. It contains experimentally determined values of human estrogen receptor alpha for both the receptor binding ability (RBA) and the transcriptional receptor gene activity (RA), both expressed as percentage of activity using estradiol as reference. According to the METI database bentazone is stated to show no activity with regard to both the human estrogen receptor alpha binding and its transcriptional activity.

3.10.3.4 Published literature 4 – hormonal activity

Kojima 2004

Previous evaluation: submitted for renewal (supplementary)

In the study 200 pesticides, including some isomers and metabolites, were tested for agonism and antagonism to two human estrogen receptor (hER) subtypes, hERα and hERβ, and a human androgen receptor (hAR) by highly sensitive transactivation assays using Chinese hamster ovary cells. In this Luciferase reporter gene assay the pesticides were evaluated for their agonistic activity based on relative activity, expressed as that concentration that showed 20% of the activity of 0.1 nM estradiol (E2), 1 nM E2 or 1 nM dihydrotestosterone (DHT) for hERα, hERβ or hAR, respectively. The pesticides, including bentazone (purity > 95%) were tested up to a concentration of 10 µM. Bentazone revealed no agonistic nor antagonistic estrogen/androgen activities up to concentrations of 10 µM in this *in vitro* Luciferase reporter gene assay using Chinese Hamster Ovary cells.

3.10.3.5 Published literature 5 – hormonal activity

Bauer 2002

Previous evaluation: submitted for renewal (supplementary)

The study describes the development and application of a receptor assay based on recombinant human androgen receptor AR (rhAR). The assay is stated to exhibit the same binding characteristics as native hAR. Bentazone (purity 99.9%, dissolved in ethanol) was tested with 28 other pesticides for its ability to displace Tritium-labelled-Dihydrotestosterone (^{3}H-DHT) bound to the rhAR, which was immobilized in microtiter place via a specific antibody. To this end, a 100 µl receptor preparation aliquot is assay buffer was incubated 16 h at 4^{o}C with 0.44 nM ^{3}H-DHT in the presence of increasing concentrations of bentazone. The receptor was fixed on the microtiter plate with a specific hAR antibody. After washing of the plates for two times with washing buffer, the specific of ^{3}H-DHT was measured as difference of total protein binding and non-specific binding in presence of a 200-fold surplus of unlabelled DHT. Bentazone showed no ^{3}H-DHT displacement even at the highest soluble concentration, indicating no binding affinity to the hAR.

3.10.3.6 Published literature 6 – hormonal activity

Bitsch 2002

Previous evaluation: submitted for renewal (supplementary)

57 active ingredients of pesticides were tested for their potential estrogenic activity using the E-Screen Assay based on the human breast cancer cell line MCF-7. Bentazone showed no estrogen-receptor mediated activity in the *in vitro* test system.

3.11 Specific target organ toxicity – single exposure

3.11.1 Animal data

Not evaluated in this dossier.

3.11.2 Human data

Not evaluated in this dossier.

3.11.3 Other data

Not evaluated in this dossier

3.12 Specific target organ toxicity – repeated exposure

Not evaluated in this dossier. However, summaries of the repeated dose studies are provided to support the evaluation of the endpoint reproductive toxicity.

3.12.1 Animal data

3.12.1.1 Study 1 – 28-day oral toxicity, rat

Anonymous 1981 (Doc. No. 81/10240)

One-month toxicity tests for bentazone in rats (tests to determine the dosage levels for 24-month toxicity tests); BASF Reg. Doc. No. 81/10240

Testing facility: date of experimental work completed: 5 January 1981

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed prior to implementation of specific test guidelines. However, to a great extent, the study was in compliance with the demands of Directive 92/69/EEC, part B, December 29, 1992.
Deviations: Clinical chemistry examinations were not included.
Additional investigations: Water consumption and organ weight of spleen, heart, pituitary, adrenal glands and ovaries were determined. Additional histopathological examination of pituitary and ovaries was carried out.
GLP: No. When the study was performed, GLP was not compulsory.
Acceptance: The study is considered supplementary only for evaluation purposes since neither raw data nor group means for the parameters measured were submitted. Thus, it was impossible to verify the information provided in the report. In addition, histopathology was not conducted in high dose rats.
Test system: Bentazone (purity: 93.9%; batch no. not available) was administered as admixture in the diet to five groups of male and female Fisher 344/CRJ rats (source: Charles River Japan) at dose levels of 0; 600; 1800; 5000 and 10000 ppm for 31 - 33 days. Eight rats per sex and group were treated. The animals were housed singly under standard conditions. Clinical observations were made twice a day. Body weight, food and drinking water consumption were recorded twice a week. Hematological, gross-pathological and histopathological examinations were carried out at the end of the administration period.
Statistics: Student's t-test.

Results

Diet analysis: The dietary concentrations of 600; 1800 and 10000 ppm were in the range of 95.3 - 103% of nominal and confirmed the intended dose level; nominal 5000 ppm ranged between 77.2 - 83.2% and corresponded to an actual concentration of about 4000 ppm, therefore.
Substance intake: tabulated below.

Table 3.12.1.1-1: Mean substance intake in rats

Dose level (ppm)	**Males (mg/kg bw)**	**Females (mg/kg bw)**
600	64	71
1800	196	217
5000 (4000)	554	607
10000	1068	1132

General observations: At 10000 ppm, male and female rats showed cyanosis of the skin of distal parts of the body including the limbs, piloerection and an incidental fading of the pigment of the fundus of the eyeball. One male rat died on day 10 of treatment. Body weight gain of male rats of the 10000 ppm dose group was significantly suppressed. A temporary decrease in food consumption was observed in both sexes. No clinical signs of toxicity were observed in animals of any other dose group.

Hematology. Clinical chemistry, Urinalysis: Hematological examination revealed a decrease in hemoglobin and hematocrit in male rats of the 10000 ppm dose group and a fall in the mean red blood cell hemoglobin while white blood cell count was significantly increased in this male group. The prothrombine (PT) and partial thromboplastin times (PTT) were significantly prolonged in both sexes at this dose level.

Pathology: Necropsy findings of the animal that died comprised subcutaneous bleeding and bleeding from the thorax and thymus gland. Necropsy of animals sacrificed at the end of the study showed bleeding in various tissues and organs in seven males and three females at 10000 ppm. Absolute weights of heart and testes were significantly decreased in male rats of the 10000 ppm dose group, while the weights of liver and left kidney were significantly increased in females of this group. Histopathological examination additionally revealed bleeding from the renal cortex in one male rat and from the ovaries of two female rats at 5000 ppm. In the two lower dose groups, no substance-related findings were observed.

Conclusion

Bentazone led to an impairment of blood coagulation in rats. Male animals were more affected than females. The bleedings observed in several organs and anemia were assessed to be related to this. The decreased absolute weight of heart and testes were attributed rather to the impaired body weight gain than to compound administration itself. Liver and kidney changes were substantiated by increased organ weight although histopathology failed to demonstrate an effect. **Under the conditions of this study, the NOEL was 1800 ppm equivalent to about 200 mg/kg bw/day for male and female animals.** This was based on toxicity apparent at the top dose level and equivocal findings like bleeding from the urogenital system in some animals at 5000 ppm.

3.12.1.2 Study 2 – 28-day oral toxicity, mouse

Anonymous (1981) (Doc. No. 81/10239)

Thirty-day oral toxicity study of bentazon in mice; BASF Reg. Doc. No. 81/10239

Testing facility: experimental work completed: 09/1980

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed prior to implementation of specific test guidelines. However, its major part is in compliance with the demands of Directive 92/69/EEC, part B, December 29, 1992.

Deviations: Hematological examinations were restricted to blood coagulation. Clinical chemistry investigations were not performed.

Additional investigations: Six animals/sex/group instead of five were used. Spleen, heart, brain, pituitary and ovaries were weighed and heart, brain, pituitary and ovaries were histopathologically examined.
GLP: No. When the study was performed, GLP was not compulsory.
Acceptance: The study is considered supplementary only since blood coagulation parameters were not determined in the low dose group. Therefore, it was not possible to establish a NOEL in this study since these changes seem to be the most sensitive indicators of compound-related toxicity. In addition, some reporting deficiencies were noted. For example, group means of body weight were not accurately calculated at some occasions. Furthermore, in the original report administration by gavage is indicated. On the same page, the actual amount of bentazone in feed is tabulated. It is assumed, therefore, that the compound was given as admixture in the diet and not by gavage.
Test system: Bentazone technical (batch no. not available) was administered to six male and female B6C3F1/CRJ mice (source: not reported) per sex and group for 30 days. Nominal dose levels were 0; 400; 2000; 5000 and 10000 ppm in the diet. The animals were individually housed under controlled conditions. Clinical observations were made daily. Body weight, food and drinking water consumption were recorded twice a week. Limited hematological, gross-pathological and histopathological examinations were carried out at the end of the administration period.
Statistics: Student's t-test.

Results

Diet analysis: The analysis of the diet revealed the following concentrations (double determination):

0 ppm	0 mg/kg feed
400 ppm	400; 400 mg/kg feed
2000 ppm	1890; 1912 mg/kg feed
5000 ppm	4703; 4747 mg/kg feed
10000 ppm	9758; 9934 mg/kg feed and confirmed that the intended concentrations were nearly achieved.

Substance intake: The mean substance intake is compiled in Table 3.12.1.2-1.

Table 3.12.1.2-1: Mean substance intake in mice

Dose level (ppm)	Males (mg/kg bw)	Females (mg/kg bw)
0	0	0
400	91	100
2000	407	487
5000	905	1004
10000	1469	1663

General observations: No deaths occurred and no changes were seen in general behavior and appearance in all mice of the 0; 400 and 2000 ppm dose groups. In contradiction, at 5000 ppm and 10000 ppm, toxic symptoms such as depression, paleness of skin and abnormal low skin temperature were noted three to five days after study had commenced. All animals of the 10000 ppm dose group died within the dosing period. At 5000 ppm, all six males and four females died. No significant differences were observed in body weight gain between animals of the 400 ppm and 2000 ppm dose groups and the control group. In contrast, the body weight gain of male and female mice of the 5000 ppm dose group was significantly suppressed when compared with the control group. At 10000 ppm, highly significant body weight losses were apparent prior to death. Food and water consumption of animals at 400 ppm and 2000 ppm were within the normal range during the study period. In male and female mice of the 5000 ppm and 10000 ppm groups, food and water consumption were significantly decreased.

Blood coagulation: The examination showed no compound-related influence on platelets count and thromboplastin-generation screening test (TST) time in any of the mice examined. However, it must be stated that it was confined to the mice surviving until scheduled sacrifice. The animals receiving the 400 ppm dose were not examined at all. At 2000 ppm, a prolongation of PTT and PT was observed in both sexes as compared to the control group. These parametes were not measured or not submitted for the two surviving female mice of the 5000 ppm dose group. However, both these females exhibited an abnormal prolongation of reaction time and clot formation time as compared to the 2000 ppm group.

Pathology: Necropsy findings of animals that died intercurrently were hemorrhagic lesions in the pia mater, subcutaneous tissue, lungs, thoracic and abdominal cavities, pericardial cavity, epicardium, thymus, orbital region and skeletal muscles. Necropsy of mice sacrificed at the end of the study revealed meningeal hemorrhage in the left cerebral hemisphere in one female of the 5000 ppm dose group, pigmentation in the spleen of one male of the 2000 ppm dose group and enlargement of the right ovary of one female of the control group which was diagnosed as follicular cyst. Histopathological examination of animals of the 5000 ppm and 10000 ppm dose groups additionally revealed hemosiderosis and extramedullary hematopoiesis in the spleen, hemorrhage and hemosiderosis in the cardiac muscles and hemorrhagic lesions in the cerebral cortex. No compound-related changes were observed in animals of the low dose group. However, the mean absolute weight of the kidneys and the relative kidney weight in male and female mice receiving the 400 ppm dose were found to be significantly higher than in the control group.

Conclusion

Repeated administration of bentazone to mice led to an impairment of blood coagulation at least at a dose level of 2000 ppm and above. The clinical signs and the hemorrhages observed at 5000 and 10000 ppm are attributable to this effect which was also seen in rats (see section 3.12.1.1). However, it must be taken into account that the latter effects occurred at lethal or sublethal dose levels only. The increased absolute and

relative kidney weights at 400 ppm are assessed as incidental since no organ weight changes were noted at higher dose levels and since histopathology did not give any indication of renal impairment at this or at the higher dose levels investigated. For this reason, the applicant had assessed 400 ppm to represent the NOAEL in this study.

However, it seems not to be justified to establish a NOEL or NOAEL at this lowest dose level since blood coagulation parameters have not been investigated and since there were effects at the next higher dose level of 2000 ppm. **Thus, a clear NOEL could not be obtained in this study.**

3.12.1.3 Study 3 – 90-day oral toxicity, rat

Anonymous 1970 (Doc. No. 70/008)

90-day feeding trial on rats with 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)-2,2-dioxide; BASF Reg. Doc. No. 70/008

Testing facility: experimental work completed: 1970

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed prior to implementation of specific test guidelines. Thus, the major part of the study is not in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.

GLP: No, study was performed prior to implementation of GLP guidelines.

Acceptance: The study is considered supplementary only since information on the test substance, in particular its purity, and on daily substance intake are lacking. A diet analysis was not conducted.

Test system: Male and female SPF breed Sprague-Dawley rats (source: Gassner, Ottobrunn, Germany) were given bentazone technical (batch no. not available) for 90 days as admixture in the diet. Dose levels were 0; 70; 200; 800 and 1600 ppm. Dose groups consisted of 20 animals per sex. Ten additional rats per sex receiving 0; 70 or 1600 ppm, respectively, were maintained for a six-week post observation (recovery) period on untreated diet. The animals were housed 5 per cage during the first 14 days of treatment and 3 or 2 per cage during the remaining period and received standardized diet (Altromin). The feed/ test substance preparations were prepared every 14 days. Clinical observations and determination of food consumption were recorded daily. Body weight was determined weekly. Hematological (red and absolute and differential white blood cell count, hemoglobin and hematocrit, reticulocytes, platelets and prothrombin time) and clinicochemical (ALAT, alkaline phosphatase and blood urea) examinations and urinalysis were carried out twice during the study. At the end of the study, rats were subjected to gross-pathological and histopathological examinations.

Statistics: Student's t-test.

Results

Diet analysis: not performed.

Substance intake: not calculated.

General observations: No clinical signs of toxicity were observed. Food consumption and body weight gain of all treated male animals were comparable with those of the control animals. At 1600 ppm, body weight gain of the female animals was slightly retarded. There were no significant differences in the absolute body weights of males and females in treated and control groups although body weights of male and female animals in the highest dose group tended to be slightly lower. Food consumption of both sexes and body weight gain of the male animals remained unaffected during the post-observation period. Females of the top dose group exhibited a lower body weight gain during this period, too.

Hematology, Clinical chemistry, Urinalysis: No treatment-related changes could be observed in hematological and biochemical examinations in test or control animals.

Pathology: There were no appreciable differences in the mean absolute weights of liver, kidneys and heart. The relative kidney weight of male animals of the 1600 ppm group and of female animals of the two upper dose groups was increased when compared with control values. Females of the 70; 800 and 1600 ppm groups exhibited increased kidney-to-heart ratios.

The relative liver weights of all treated animals did not differ from those of the controls. However, at 1600 ppm, male animals showed an increased relative liver-to-heart weight ratio. In addition, heart weights and the kidney-to-heart weight ratio were higher than those of the controls in this group. Female animals of the 70 ppm group exhibited lower relative heart weights and the liver-to-heart weight ratio was increased at this dose level. In the withdrawal groups, all these increased ratios proved to be reversible.

No test substance-related macroscopic changes were found at necropsy of the test animals. Two males of each of the 200 ppm and 1600 ppm groups were found to have detectable degeneration of the testicular tissue. No further histopathological changes occurred in any of the other organs.

Conclusion

Under the conditions of this study, **the NOEL is assessed to be 200 ppm (corresponding to about 10 mg/kg bw/day) for male and female animals.** This value is based on an increased relative kidney weight in females at 800 ppm and slight effects on body weight gain at the top dose level. Although no histopathological changes were observed, the liver weight changes were assessed as indicative for a slight liver adaptation process induced by the administration of the test substance.

The minor and inconclusive effects on organ weights noted at 70 and 200 ppm in single animals are assessed as incidental because there was a lack of a dose- response relationship. Similarly, the fact that only two male animals of each of the 200 ppm and 1600 ppm groups were found to have histopathologically detectable degeneration of the testicular tissue and that such changes were not observed up to the highest dose (3600

ppm) in the subchronic study described below (section 3.12.1.2) might indicate that this finding was not test substance-related.

3.12.1.4 Study 4- 90-day oral toxicity, rat

Anonymous 1987 (Doc. No. 87/0173)

13-week oral toxicity (feeding) study with bentazon technical (ZNT No. 86/48) in the rat; BASF Reg. Doc. No. 87/0173

Testing facility: date of experimental work completed: 24 July 1986

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed according to OECD guideline 408, EPA/FIFRA, Subdivision F, 82-1 and is therefore in compliance with the demands of Directive 87/302/EEC, part B, May 30, 1988.
Deviations: None.

GLP: Yes. When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP. A formal GLP Compliance and a Quality Assurance Statement are included in the report.

Acceptance: The study is considered acceptable.

Test system: Male and female Wistar rats (KFM-Han., outbred, SPF quality; source: Madoerin AG, Fuellinsdorf, Switzerland) were administered bentazone technical (Batch no. N 187; purity 97.8%) for 90 days in the diet. Groups of 10 animals per sex received the compound at dose levels of 0; 400; 1200 and 3600 ppm. The reversibility of treatment-related changes was studied with 10 additional animals per sex in the control and the highest dose group over a four-week recovery period. The animals were housed 5 per cage under controlled conditions and had free access to standardized diet. Tap water was available ad libitum. The test substance/feed preparations were prepared twice monthly and stored at room temperature. Clinical observations were made twice daily. Food consumption and body weight were recorded weekly. Clinicochemical, hematological, ophthalmological, gross-pathological and histological examinations were carried out after 13 weeks for the main study groups and after 17 weeks for the recovery groups .

Statistics: For body weights, food consumption, organ weights and clinical laboratory data, univariate one-way analysis of variance was conducted followed by Dunnett-test or Steel-test. For the overall spontaneous mortality data, Fisher's exact test was applied.

Results

Analysis: The analysis confirmed the stability for at least 21 days in the diet, the homogenicity in the diet and the intended concentrations.

Substance intake: Dose levels of 0; 400; 1200 and 3600 ppm in the diet corresponded to daily intake levels of 0; 25.3; 77.8 and 243.3 mg/kg bw for male animals and to 0; 28.9; 86.1 and 258.3 mg/kg bw for female animals.

General observation: There were three cases of intercurrent death. Two animals of the 3600 ppm group were found dead in their cages during the 9th and 12th week of treatment, respectively. Their cause of death could not be determined. However, it is not likely that these deaths were attributable to treatment since there were no preceding signs of toxicity noted in these rats. One female of the 3600 ppm group died during anesthesia on the day of scheduled necropsy. No signs of toxicity were seen in any dose group. Food consumption was similar in all groups of males and females, respectively. At 3600 ppm, mean body weight gain was slightly reduced for females leading to a 6% reduction (significant at 5% level) in mean terminal body weight relative to controls after 13 weeks of treatment. Body weight gain noted in males of this dose group and for both sexes at the 400 ppm and 1200 ppm dose levels was similar to the respective control groups. Body weight gain of animals of the 3600 ppm recovery group during the 4-week regression period was in its mean also.similar to that of the respective control animals. Ophthalmoscopy revealed no substance-related effects.

Hematology, Clinical chemistry, Urinalysis: Hematological examinations showed prolonged thromboplastin and partial thromboplastin times for male animals of the 3600 ppm group. The prolonged coagulation times may reflect an inhibitory effect on blood clotting factors. This effect was found to be reversible at the end of the recovery period. The biological meaning of a shortened prothrombine time as seen in females is equivocal.

Table 3.12.1.4-1: Blood coagulation parameters in rats (males/females) after 13 or 17 weeks, respectively

Dose (ppm)	**0**	**400**	**1200**	**3600**	**0 (recovery)**	**3600(re-covery)**
PT (sec)	13.5/ 13.2	13.2/ 12.5*	13 .2/ 12.4*	15.8*/ 12.4*	13.3/ 12 .9	13.4/ 13.0
PTT(sec)	22.5/ 20.4	22 .3/ 21.3	23.9/ 20.8	30.2*/ 21.8	21.2/ 19.2	21.4/ 18.9

* statistically significant at 5% level

PT : prothrombine time

PTT: partial thromboplastin times

Clinical biochemistry revealed an increased total cholesterol level for females of the 3600 ppm group, as well as an increased albumin fraction and albumin to globulin ratio (A/G-ratio) for males of the 1200 ppm and 3600 ppm groups. These changes were found to be reversible at the end of the treatment-free period. Urinalysis data indicated an increased urinary output (volume/18h) reaching significance for both sexes at 3600 ppm and a corresponding decrease in specific gravity.

Table 3.12.1.4-2: Clinical chemistry and urinalysis findings in rats after 13 or 17 weeks, respectively

Dose (ppm)	0	400	1200	3600	0 (recovery)	3600(re-covery)
Cholesterol (mmol/l)	2.36/ 2 .30	2.49/ 2.45	2.32/ 2.45	2.49/ 2.65*	2.48/ 2.30	2.27/ 2.47
Albumin (g/D	34.6/ 43.0	34.7/ 41.4	35.5/ 43.4	36.4*/ 42.1	40.6/ 47.2	39.6/ 46.2
A/G -ratio	1.02/ 1.59	1.02/ 1.46	1.12*/ 1.49	1.19*/ 1.51	1.13/ 1.45	1.11/ 1.44
Urine volume/ 18 h (ml)	4.7/ 4.9	5.2/ 6.2	5.5/ 6.3	7.5*/ 6.7*	8.1/ 8.9	9.4/ 7.0
Specific gravity	1.054/ 1.039	1.046/ 1.030*	1.046/ 1.028*	1.036*/ 1.029*	1.033/ 1.021	1.029/ 1.027*

* statistically significant at 5% level

Pathology: A slight enlargement of the kidneys was noted macroscopically for both sexes at 3600 ppm. This effect was more marked in males than in females, however, it was found fully reversible after the withdrawal period in male rats only but not in females. No further gross-pathological changes were detected and histopathology did not demonstrate any effect.

Table 3.12.1.4-3: Absolute kidney weight (g) and kidney/body weight ratio (%) in rats after 13 or 17 weeks, respectively

Dose (ppm)	0	400	1200	3600	0 (recovery)	3600(re-covery)
Males ratio	2.03 0.55	2.22 0.56	2.01 0.54	2 .44** 0.63**	2.16 0.53	2.21 0.57
Females ratio	1.34 0.59	1.34 0.59	1.43 0.62	1.44 0.67**	1.32 0.57	1.44 0.65*

* statistically significant at 5% level ** statistically significant at 1% level

Conclusion

The predominant finding was an impairment of blood coagulation. Additionally, minor effects in clinical chemistry (1200 ppm and above) and renal function (3600 ppm) were recorded. The minor effects at 1200 ppm (slight increase in A/G ratio in males and decreased PT in females) is not considered to be advers. Kidney weight was increased at the top dose level at least in males. Based on these findings, **the NOEL was**

1200 ppm equivalent to 77.8 mg/kg bw/day for male and 86.1 mg/kg bw/day for female animals in this study.

3.12.1.5 Study 5 – 90-day oral toxicity, rat

Characteristics

reference	:	Anonymous 2011 (Doc No. 2011/1173365) Anonymous 2012 (Doc No. 2012/1009658)	exposure	:	90 days, in diet
type of study	:	90-day oral toxicity study	doses	:	Bentazone-sodium (BAS 351 H-Na) 0, 475, 1425 and 4275 ppm[1] Bentazone acid (BAS 351 H) 3600 ppm[2]
year of execution	:	2011	vehicle	:	None
test substance	:	BAS 351 H-Na, batch no. COD-001417, purity 91.9% BAS 351 H, batch no. COD-001416, purity 100%	GLP statement	:	yes
route	:	oral	guideline	:	in accordance with OECD 408
species	:	Rat, Crl:WI	acceptability	:	acceptable
group size	:	/sex/dose	**NOAEL**	:	1425 ppm (91 mg/kg bw/d)
			Previous evaluation		Submitted for renewal (supplementary)

[1] Equal to 0, 31, 91 and 290 mg/kg bw/d for males and 0, 42, 98, 304 mg/kg bw/d for females.

[2] Equal to 238 mg/kg for males and 252 mg/kg bw/day for females.

Study design

The study was performed in accordance with OECD 408. The aim of the study was to check for toxicological equivalence of Bentazone-sodium, which is the manufactured use product, to Bentazone-acid. For bentazone-sodium doses tested were selected to be equimolar to doses tested in the 13-week rat oral toxicity study (Anonymous 1987, section 3.12.1.4) (0, 475, 1425, 4275 ppm, equivalent to 31, 91 and 290 mg/kg bw/day for males and 42, 98 and 304 mg/kg bw/day for females). For bentazone-acid 3600 ppm (238 mg/kg bw/day in males, 252 mg/kg bw/day in females) was tested which is equimolar to 4275 ppm bentazone-sodium.

Results

Table 3.12.1.5-1: Mean compound intake over the duration of the study

Test group	**Concentration in the diet (ppm)**	**Mean daily test substance intake (mg/kg bw/day)**		**Mean daily test substance intake calculated equimolar (mg/kg bw/day)**	
		Males	**Females**	**Males**	**Females**
1	475 ppm bentazone-	31	42	26	35

	sodium				
2	1425 ppm bentazone-sodium	91	98	76	82
3	4275 ppm bentazone-sodium	290	304	244	256
4	3600 ppm bentazone-acid	238	252	238	252

Table 3.12.1.5-2:

	Bentazone-sodium								Bentazone-acid	
Dose (mg/kg food)	**0**		**475**		**1425**		**4275**		**3600**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities occurred.									
Clinical signs	No treatment-related findings.									
Body weight			-0.2%	-0.3%	-0.3%	+0.5%	-6.6%	-3.7%	-4.5%	-4.2%
Body weight gain			-0.6%	-0.3%	+0.2%	-0.1%	-10.6%	-10.5%	-7.7%	-10.7%
Food consumption	No treatment-related findings.									
Functional observational battery	No treatment-related findings.									
Ophthalmoscopy	No treatment-related findings.									
Haematology										
- PTT	19.9	20.7	19.8	20.1	19.9	19.5	**23.0****	20.7	**22.4****	20.9
- QT	18.0	17.5	17.6	16.9	18.1	**16.3***	**20.2***	**16.3***	**19.8***	17.2
- MCH	1.02	1.11	1.01	1.08	1.04	1.09	1.01	1.09	1.04	**1.08***
- MCHC	20.22	20.43	20.19	20.35	20.39	20.14	20.13	20.23	20.31	**20.20***
Urinalyses										
- spec. gravity	1.038	1.037	1.039	**1.055***	1.035	1.038	1.036	1.035	**1.033***	1.038
- pH	6.16	5.68	6.31	5.70	6.29	5.69	6.25	**5.38****	6.34	5.54
Clinical chemistry										
-Globuline	27.40	25.42	26.16	25.58	25.86	26.12	25.38	**24.62***	25.22	**24.38***
-Cholesterol	1.77	1.51	1.88	1.67	1.80	1.78	1.95	1.73	1.91	**1.98***
-Trigliceryde	0.72	0.37	0.69	0.44	0.88	0.38	0.85	0.49	0.93	**0.56***

Organ weights										
Adrenal										
-Absolute	61.6	74.3	57.0	73.8	57.8	76.2	59.5	69.4	60.2	**67.0***
-Relative	0.016	0.035	0.015	0.035	0.015	0.035	0.016	0.035	0.016	0.033
Brain										
- Absolute	2.057	1.934	2.057	1.894	2.06	1.925	2.085	1.926	2.079	1.913
- Relative	0.529	0.919	0.529	0.899	0.526	0.899	**0.573***	0.956	0.557	0.95
Heart										
- Absolute	1.06	0.674	1.065	0.695	1.043	0.695	0.966	0.686	1.003	0.69
- Relative	0.271	0.32	0.272	0.328	0.267	0.323	0.265	0.34	0.268	**0.342***
Kidneys										
- Absolute	2.38	1.346	2.32	1.367	2.268	1.422	2.416	**1.622****	2.451	**1.587****
- Relative	0.609	0.638	0.595	0.646	0.579	0.66	**0.662***	**0.806****	0.655	**0.786****
Liver										
- Absolute	8.734	4.854	8.706	5.122	8.128	5.074	8.038	5.014	8.351	4.977
- Relative	2.224	2.302	2.231	2.419	2.072	2.359	2.202	**2.481****	2.232	**2.467****
Thymus										
- Absolute	0.312	0.259	0.34	0.269	0.312	0.271	0.305	0.245	0.301	**0.295***
- Relative	0.08	0.123	0.088	0.128	0.079	0.127	0.083	0.121	0.08	**0.146***
Pathology										
macroscopy	No treatment-related findings									
Microscopy										
- nephropathy	5	4	6	0	8	0	10	4	9	1
Grade 1	4		6		6		3		5	
Grade 2	1		0		2		7		4	

*p ≤ 0.05, **p ≤ 0.01

Dietary administration of bentazone-sodium at 4275 ppm and bentazon-acid at 3600 ppm did not result in any significant changes in body weight in male and female animals compared to controls. However, there was a trend to reduced body weights and body weight gain in both sexes at 4275 ppm bentazone-sodium and 3600 ppm bentazone-acid towards the end of the study.

In addition, prolonged activated partial thromboplastin time (PTT) and prothrombin time (QT) in males and decreased globulin values in females were observed at the highest dose. In females at 3600 ppm bentazone-acid lower mean corpuscular hemoglobin content (MCH) and mean corpuscular hemoglobin concentrations (MCHC) compared to controls were calculated. Because no measured red blood cell parameter (hemoglobin, red blood cell counts, hematocrit), was changed, these alterations were regarded as incidental and not treatment-related. At the end of the study, in females at 3600 ppm bentazone-acid globulin levels were decreased, whereas cholesterol, triglyceride and potassium mean concentrations were increased. In females at 4275 ppm bentazone-sodium globulin levels were also decreased.

Kidney (absolute and relative) weights and relative liver weights were increased in females without histopathological findings. With respect to the high dose males only the relative kidney weights were increased. The increase of relative brain weight in males and relative heart weights in females was

considered secondary to the reduced terminal body weight and thus not of toxicological relevance. In the absence of any corroborative histopathological findings the increase in thymus weights in bentazone-acid treated females is regarded as unlikely to be treatment related. This is supported by the previous 90-day toxicity study with bentazone-acid (section 3.12.1.4) where no effect on the thymus was observed.

A treatment-related increase in incidence of minimal to slight chronic nephropathy was noted in male animals at 1425 and 4275 ppm bentazone-sodium and 3600 ppm bentazone-acid. These findings were regarded as non-adverse as the severity was not increased over the grading already observed in control animals. All other histopathological findings were either single observations, were equally distributed between control and treated groups or displayed no dose-response relationship.

Acceptability

The study is considered acceptable.

Conclusion

The bentazone group showed similar results compared with the 4275 ppm bentazone-sodium group and thereby demonstrated the equivalence of both substances. Furthermore, the results were comparable with the findings in the previous 90-day toxicity study the rat (section 3.12.1.4). Based on the haematological findings and the increase in absolute and relative organ weights at 4275 ppm the NOAEL is set at 1425 ppm, equivalent to 91 mg/kg bw/day in males and 98 mg/kg bw/day in females.

3.12.1.6 Study 6 – 90-day oral toxicity, dog

Anonymous 1970 (Doc. No. 70/009)

13-week toxicity of 3-isopropyl-lH-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide (hereafter referred to as XIX/410) to beagles when administered with the food; BASF Reg. Doc. No. 70/009

Testing facility:; experimental work completed: 07/1970

Previous evaluation: in original DAR

Material and methods

Test method: This study was performed prior to implementation of specific test guidelines. However, to a great extent, the study fulfilled the demands of Directive 87/302/EEC, part B, May 30, 1988.

Deviations: The extent of clinical chemistry and of histopathology shows minor deviations to the requirements of the Directive 87/302/EEC, part B, May 30, 1988. Diet analysis was not performed.

GLP: No. The study was performed prior to implementation of GLP guidelines.

Acceptance: With respect to the year when it was performed, the study is considered acceptable.

Test system: Bentazone technical (batch no. not available) was given to male and female Beagle dogs (source: Laboratory of Pharmacology and Toxicology, Hamburg) for three months as admixture in the diet. Dose

levels were 0; 100; 300; 1000 and 3000 ppm. The test substance/feed preparations were prepared fresh daily. Test and control groups consisted of three dogs per sex each. The animals were housed singly under controlled conditions and received a daily ration of 40 g/kg body weight commercial diet (Altromin H). Water was available ad libitum. Clinical observations, food and drinking water consumption were recorded daily. Body weight was determined weekly. Hematology, clinicochemical examinations and urinalysis were carried out before the administration period commenced and after 6 and 13 weeks of treatment. A sight and hearing test, ophthalmoscopy, gross-pathological and histopathological examinations were carried out at the end of the study.
Statistics: Student's t-test.

Results
Substance intake: Dose levels of 0; 100; 300; 1000 and 3000 ppm in the diet corresponded to mean daily intake levels of 0; 4.0; 12.0; 39.6 and 113.8 mg/kg body weight for both sexes.

General observations: Daily doses of 100 ppm and 300 ppm were tolerated without any symptoms.
At 1000 ppm, one male dog displayed a slight but increasing sedation during the last two weeks of the study. The same dog developed an ulcer on the left hind leg. The surrounding area was affected by alopecia, and the ulcer had not healed at the end of the study in spite of cleansing with normal saline. No other pathological changes were noted in any other dog at this dose level. At 3000 ppm, three of the six animals (one male, two females) died in coma. Signs of toxicity noted were sedation, attacks of hyperactivity, ataxia, prostration, loss of rising reflex and tremors. The sedative effect was seen first between the 2nd and 4th week of treatment in all high dose dogs. It appeared 10 to 60 minutes after food and compound intake. Its duration increased from about 5 hours up to 24 hours at the end of the treatment period. The three male dogs in this group vomited from time to time. In the second half of the study, all animals had increasingly severe diarrhoea, in some cases with visible blood. Anorexia was observed during the whole study period. At first, the food consumption was only retarded, but later the amount consumed was reduced. All animals lost weight. At 3000 ppm, all six animals had bilateral hemorrhagic conjunctivitis, mostly in a mild form. Male animals exhibited ulcerative stomatitis. One male animal had ulcerations surrounded by areas of alopecia on the right paw, the right ear and left of the umbilicus. The recuperative powers of the animals appeared to have been diminished since none of these inflammatory changes healed by the end of the trial. Oedema in the thoracic region was observed in one male dog.

Hematology. Clinical chemistry. Urinalvsis. Erythrocyte count, hemoglobin and hematocrit were reduced in the highest dose group. Blood sedimentation and blood coagulation were retarded in this group and the platelet count was reduced. Furthermore, there was increased activity of several serum enzymes like alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase, increased urea and

bilirubin contents and reduced albumin and total protein concentrations in the blood. Albuminuria and ketonuria occurred more frequently in this group.

Pathology: At 3000 ppm, necropsy in all dogs revealed pale liver. Pale kidneys were noted in one dog. The males also had heavily marked lobes of the liver, and one had gastric ulcers. Swelling of the thoracic region was noted in one male and one female animal. Liver, kidneys and adrenals were distinctly enlarged. Relatively high weights were also recorded for spleen, lungs, thymus, thyroid and brain. At histopathology, there were some compound-induced changes mainly consisting of severe congestive symptoms and necrotic congestion of the liver lobe centres. Marked fatty degeneration was observed in five dogs. These liver findings were in keeping with hypoxic damage to liver parenchyma. Furthermore, extramedullary hemopoiesis in the spleen was noted. However, the bone marrow had shown a normoplastic picture. Droplets of fatty degeneration in the ventricular myocardium and the albuminous swelling of the renal tubules were observed. Histological examination of the organs from the animals of the lower dose groups revealed no compound-induced pathological changes.

Conclusion

The highest dose level of 3000 ppm was severely toxic to the dogs and was shown to be lethal in three out of six animals. The MTD was clearly exceeded. Anemia and disturbed blood coagulation as well as signs of kidney and liver damage were noted confirming the findings obtained in other species. The clinical findings like cachexia, conjunctivitis, stomatitis, oedema are assessed to be secondary effects of the severe intoxication. The histopathological findings were assumed to reflect chronic hypoxidosis caused by treatment.

The dose level of 1000 ppm represented the low observed effect level (LOEL) in this study although clinical findings were noted in one animal only. It remains equivocal whether these findings were treatment-related or not. However, the sedative effect observed was also described for all top dose animals and so were ulcerative changes with a retarded healing process. It should be mentioned that no further changes were observed in all other investigations at this or the lower dose levels. However, a particular sensitivity of some individuals cannot be excluded.

The NOEL was established at 300 ppm equivalent to 12 mg/kg bw/day for male and female dogs in this study.

3.12.1.7 Study 7 – 1-year oral toxicity, dog

Anonymous 1989 (Doc. No. 89/0049) / Anonymous 1989 (Doc. No. 89/0153)

52-week oral toxicity (feeding) study with bentazone technical (ZST No.:86/48) in the dog; BASF Reg. Doc. No. 89/0049

Testing facility:; date of experimental work completed: 2 6 November 1987

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed according to OECD guideline 452, the EPA/FIFRA, Subdivision F, guideline 83-1 for chronic toxicity studies and the "Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration" (MAFF, Japan, 59 NohSan No. 4200) and is therefore in compliance with the demands of Directive 87/302/EEC, May 30, 1987.

Deviations: None.

GLP: Yes. When the study was performed, GLP was not compulsory. However, it is stated that the study was run according to the principles of GLP. A formal GLP Compliance and a Quality Assurance Statement are included in the report.

Acceptance: The study is considered acceptable.

Test system: Bentazone technical (purity: 97.8%, batch no. N 187) was fed as admixture in the diet to male and female Beagle dogs (source: Madoerin AG, Fuellinsdorf, Switzerland) for 52 weeks at dose levels of 0; 100; 400 and 1600 ppm. Six dogs per sex and group were used. The animals were housed singly under controlled conditions and received each 300 g of standardized diet for 3 hours daily. Tap water was available ad libitum. The test substance/feed preparations were formulated twice monthly. Clinical observations and feed consumption were recorded daily. Body weight was determined weekly. Hematology, clinical biochemistry, urinalyses and ophthalmoscopy were carried out before treatment commenced and after 13, 2 6 and 52 weeks. At week 14, feces of the top dose and control animals were investigated for occult blood. A hearing test was performed prior to and at 52 weeks of treatment. At the end of the study, the animals were subjected to gross-pathological and histological examination.

Statistics: For body weights, feed consumption, organ weights and clinical laboratory data, univariate one-way analysis of variance, the Dunnett-test or the Steel-test were applied.

Results

Analysis: The analytical examinations confirmed the stability in the diet for 21 days. The analysis of the dietary concentrations confirmed the intended dose levels (100 ppm: 97.4% of theoretical value; 400 ppm: 101.0% of theoretical value; 1600 ppm: 100.1% of theoretical value (means)).

Substance intake: as shown below (Table 3.12.1.7-1).

Table 3.12.1.7-1: Mean substance intake

Dose level (ppm)	**Mean substance intake (mg/kg bw)**	
	Males	**Females**
100	3.04	3.29
400	13.07	13.2

1600	49.72	54.83

General observations: No animal died during the study period. Abnormal clinical signs were recorded for three males (nos. 20, 21 and 22) and one female of the 1600 ppm group. These signs included slight, but persistent, weight losses in the first half of the treatment period and an emaciated appearance and dehydration from week 19 to termination in dog no. 20 and transient skin lesions (hyperemia and alopecia) in male no. 22. Diarrhoea was recorded throughout the treatment period for dog no. 21. On four occasions, between days 27 and 40, the diarrhoea was thought to contain blood and subsequently the dog showed a transient decrease in activity and pallor of the mucous membranes. Marked anaemia was evident in this animal during week seven and an appreciable increase in red blood cell parameters was seen in week eight following a 6-day treatment-free period. During week three a marked reduction in food consumption and the presence of diarrhoea thought to contain blood were recorded for female no. 47. Gastroenteritis was diagnosed and the dog treated with antibiotics. An improvement in condition followed and normal food consumption was recorded from week eight. Food consumption of the remaining treated animals was unaffected. Body weight loss was evident in two males (nos. 20 and 21) and in two females (nos. 44 and 47) of the 1600 ppm group during the first five weeks of treatment. With the exception of male no. 20, which had an overall weight loss of 1.2 kg, recovery was evident in all cases. Body weight gain in the remaining treated and control dogs was similar. Auditory perception and ophthalmoscopy were unaffected by treatment.

Hematology, Clinical chemistry. Urinalysis: In general, examination of group mean hematological, biochemical and urinalysis data recorded at 13, 2 6 and 52 weeks did not reveal any findings of toxicological significance. Fecal occult blood was not detected at an examination of the dogs of the control and high dose group during week 14.

However, the following changes were noted in individual dogs of the 1600 ppm group: During week seven a deterioration in clinical condition was apparent for male no. 21 and a sample of blood was taken for hematological investigations. Its examination revealed a marked anemia, leukocytosis, enhanced erythropoietic and thrombopoietic activity of the bone marrow with a slight increase in partial thromboplastin time. A progressive recovery was evident at an additional investigation in week eight (following a 6-day period without treatment) and in week 13. Slight anemia with enhanced erythropoietic activity of the bone marrow and increased prothrombin and partial thromboplastin times was also apparent for female no. 47 in week 13.

Pathology: Organ weights were unaffected by treatment. All pathological findings recorded, including some minor testicular alterations in five dogs of different dose groups which all received the test substance, were of a spontaneous nature common to dogs of this age and strain. There was no evidence of abnormal histopathological findings resulting from treatment with bentazone.

Conclusion

Two female and two male animals in the highest dose group exhibited a reduction in the body weight that was reversible after withdrawl of treatment in two female and one male animal and correlated only in one female animal with a decreased food consumption. Two of these animals had bloody diarrhea transiently. These animals were also found to have anemia, leukocytosis and an increased blood coagulation time. Transient anemia, leukocytosis and an increased blood coagulation time were also observed in another male animal. A substance-induced effect has to be assumed. The pattern of findings suggests a particular vulnerability of some dogs. The other clinicochemical, hematological, ophthal-mological, gross-pathological and histopathological investigations did not reveal any substance-induced changes in any dose group. Based on the results of this study, **the NOEL was 400 ppm equivalent to approximately 13.1 mg/kg bw/day for male and female animals.**

3.12.1.8 Study 8 – 21-day dermal toxicity, rabbit

Anonymous 1993 (Doc. No. 93/10760)

Study of the dermal toxicity of Reg. No. 51 92 9 in White rabbits, application to the intact skin over 3 weeks; BASF Reg. Doc. No. 93/10760

Testing facility: date of experimental work completed: 27 February 1992

Previous evaluation: in original DAR

Material and methods

Test method: The study was performed according to OECD guideline 410 and its major part is therefore in compliance with the demands of Directive 92/69/EEC, part B, December 29, 1992.

Deviations: Duration of treatment was 21 days only instead of 28. No recovery period was included and histopathological examination of spleen, heart and testes was not carried out. However, with respect that bentazone caused neither local nor systemic toxicity in this study as well as in other experiments described below, it is assessed as acceptable that its duration was shorter than required and that no recovery period was implemented. It is very unlikely that the prolongation to 28 days would have led to any effects and that the examination of spleen, heart and testes would have shown any impairments of these organs.

GLP: Yes.

Acceptance: The study is considered acceptable.

Test system: Bentazone (purity: 97.64%, batch no. N 194) was administered dermally to male and female SPF New Zealand White rabbits (source: Dr. Thomae GmbH, Biberach, Germany) for 21 consecutive days at dose levels of 0 (solvent control); 250; 500 and 1000 mg/kg bw. Five animals per sex and group were tested. The test material was applied for six hours per day as 0.5% aqueous Tylose CB 30.000 solution (cleaned Na-CMC in aqua bidest.) under semi-occlusive dressing covering at least 10% of body surface. The

animals were housed singly under controlled conditions and received each a daily ration of about 130 g of standardized diet. A daily ration of approximately 250 ml/animal was available as drinking water. The test substance preparations were made up each workday immediately before application. Clinical observations were made twice daily. A check for skin findings was carried out daily about 30 min after removal of the dressing. Food consumption and body weight were recorded weekly. Clinicochemical, hematological (including clotting analysis for thromboplastin time), gross-pathological and histopathological examinations were carried out at the end of the study.

Statistics: For body weight, hematology (except differential blood count), clinical chemistry and pathology (organ weights) data, a non-parametric one-way analysis using the Kruskal-Wallis test or the Mann-Whitney-U test was performed.

Results

Analysis: The analysis of the test substance confirmed the stability for the study period, homogenicity and purity. The analysis of the preparations confirmed the stability for at least 16 hours, the homogenicity and the intended concentrations.

General observations.

Dermal findings: There were no deaths during the study period. No treatment-related differences in food consumption were noted during the study. Body weight in test animals was comparable with that seen in controls. There were no clinical signs in any test group which could be related to the test substance application. No signs of irritation on the treated skin could be observed in the test animals. The treated skin of the test animals was discolored by the test substance. Adhesive tape caused mechanical skin lesions beside the treated area.

Hematology, Clinical chemistry: No treatment-related effects in clinical chemistry and hematology values were apparent in males and females.

Pathology: No pathomorphologic findings considered to be treatment-related were diagnosed. Neither treatment-related significantly different mean absolute or relative organ weight parameters nor treatment-related gross lesions or microscopic findings were detected. No treatment-related skin changes were noted.

Conclusion

No treatment-related local and systemic toxicity could be observed. The **NOEL for the dermal toxicity (local and systemic effects)** under the conditions chosen was **1000 mg/kg bw for male and female rabbits,** therefore.

3.12.1.9 Study 9 - 21-day dermal toxicity, rabbit

Anonymous 1988 (Doc. No. 88/0350)

Study on the dermal toxicity of Reg. No. 51 929/bentazone in rabbits, application to the intact skin for 3 weeks (21 applications); BASF Reg. Doc. No. 88/0350

Testing facility:; date of experimental work completed: 1 October 1987

Acceptance: The study is considered unacceptable since the animals were infected with coccidia. It is not described in detail here, therefore. Two females of the top dose group (1000 mg/kg bw) and one female of the control group died due to this infection. Histopathology revealed chronic proliferative cholangitis of all animals (test group animals and control animals) indicative for this disease. Although neither local nor systemic effects which could be attributed to bentazone treatment were observed up to the highest dose level of 1000 mg/kg bw, the study was repeated (see section 3.12.1.8 above) using SPF rabbits since an interference with coccidiosis is unlikely but cannot be totally excluded. Findings due to this infection could have obscured toxic effects caused by the test compound.

Previous evaluation: in original DAR

3.12.1.10 Study 10 – 21-day dermal toxicity, rabbit

Anonymous 1971 (Doc. No. 71/005)

21-day toxicity of 3-isopropyl-lH-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide to NZW rabbits on local application; BASF Reg. Doc. No. 71/005

Testing facility:, date of test report: 15 March 1971

Previous evaluation: in original DAR

Material and methods

Test method: This study was performed prior to implementation of specific test guidelines. Thus, the major part of the study is not in compliance with the demands of Directive 92/69/EEC, part B, December 29, 1992.

GLP: No. The study was performed prior to implementation of GLP guidelines.

Acceptance: The study is considered supplementary only due to the lack of test material analysis for purity and concentration and due to reporting deficiencies.

Test system: Four groups of six male and six female New Zealand White rabbits (source: Laboratory of Pharmacology and Toxicology, Hamburg, Germany) with intact or scarified skin were dermally administered bentazone technical (purity not reported; batch no. not available) as 1% aqueous tylose preparation for 21 consecutive days. Dose levels of 0; 250; 500 and 1000 mg/kg bw were applied for 8 hours per day under semi-occlusive dressing covering about 10% of body surface. The animals with intact skin (i.e. half of the total) were kept under observation for a further 21 day recovery period after termination of treatment. The

animals were housed singly under controlled conditions and received standardized diet and drinking water ad libitum. Clinical observations, feed and water consumption were recorded daily. Body weight was determined weekly. Hematological examinations and urinalysis were carried out before the start and after 21 days of treatment. Biochemical examinations, a hearing test, ophthalmoscopy, gross-pathological and histopathological examination were performed at the end of the study.
Statistics: Student's t-test.

Results

Dermal findings: Very slight, transient erythema was detected on the intact and scarified skin, the latter being slightly more affected. However, in no case did the reactions exceed those seen in the control animals. More extensive skin injuries, such as edema or necrosis, were not observed. The test and control animals showed no differences during the withdrawal period.

General observations, Hematology, Clinical chemistry. Pathology: Behavior, condition of coat, food and water consumption, body weight gain, results of hematological and biochemical tests or urinalysis, gross-pathological findings and organ weights at necropsy after three or six weeks on study were similar for treated and control animals. Histological investigations carried out after three weeks on the animals with scarified skin and after a further three weeks observation period on the animals with intact skin revealed in isolated cases negligible inflammatory infiltration as well on the application site as on the untreated skin. No significant differences could be observed between the treated animals and the control animals.

Conclusion

Under the conditions of this study, **the NOEL for the dermal and systemic toxicity is 1000 mg/kg bw for male and female rabbits.**

3.13 Aspiration hazard

3.13.1 Animal data

No data.

3.13.2 Human data

No data.

3.13.3 Other data

No data.

4. ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

Main notifier data/data from original DAR

A study to test the ready biodegradability of bentazone was not performed. The substance has to be considered to be not readily biodegradable according to OECD guideline 301.

Second notifier data

The second notifier submitted a study with their renewal dossier that seems to be the same study that was originally evaluated in the DAR. An evaluation of the study to current knowledge and approache is provided below.

STUDY IIA, 7.7/01

Characteristics

reference	:	Hoek, van der E & J.F. Kreuk (1987)	incubation time	:	117 d
study type	:	biodegradability	nominal concentration	:	10 mg/L and 20 mg/L
year of execution	:	1987		:	
GLP statement	:	yes		:	
guideline	:	OECD 301B	Conclusion	:	See below
test substance	:	Bentazone technical	acceptability	:	**supplemental**
purity	:	97.5%	Previous evaluation	:	Submitted for renewal (supplementary)

Study design

Ditch-water and ditch-sediment were taken from a ditch surrounding the premises of the TNO Zuidpolder complex in Delft, the Netherlands. 150 mg sediment was added on a dry weight basis per litre ditchwater. Amounts of sediment corresponding to 150 mg dry matter were placed in nine two-litre bottles, with one litre of ditch-water and aerated with C0?-free air for 24 hours before addition of the test substance. The test was carried out in triplicate; two concentrations of test substance (10 and 20 mg.l respectively) being prepared by adding the correct amount of dry test substance to each of three bottles; the remaining three bottles

constituted the blank. The bottles were incubated for 117 days on a Shake orbital shaking machine in a room kept at 20 °C ± 2°C.

The $C0_2$-absorption vials placed in the screw cap of the bottle, were replaced by fresh ones after 7, 14, 21, 42, 56, and 84 days and the amount of CO_2 absorbed determined according to OECD 301 B (1) by titration with 0.1 M HCl. The average background CO_2-production by the blank bottles was substracted from the production in the other bottles; this corrected $C0_2$-production was used to calculate the biodegradability.

A control experiment was carried out to determine the inoculum activity and to detect possible toxic effects of the test substance using 6 additional bottles prepared as described above; to each of two bottles such quantities of Bentazone were added that concentrations of 0, 10 and 20 mg.l were obtained. In this control experiment sodium acetate was added to the inoculated medium as a carbon source (100 mg.l , theoretical (CO_2 -production 1.07 mg/L). The results of the control test were corrected for the background CO_2 production by the sediment as described above.

A parallel experiment was conducted to allow chemical analysis of Bentazone; bottles contained concentrations of 0, 10 and 20 mg.l . At the beginning of the experiment, after four weeks and at the end of the experiment, the concentration of Bentazone was determined in duplicate bottles with the aid of gas chromatography after preparation of the methyl derivative. The complete contents of one flask were used for one analysis. The biodegradation after four weeks and at the end of the experiment was estimated by comparison of the Bentazone concentrations measured at those times with those measured at the beginning of the experiment.

Results

The biodegradability of acetate was used as a measure for the microbial activity of the sediment and its possible inhibition by the presence of the test substance. Results show that 43% of the acetate was transformed to CO_2 during the first 2 weeks. No effect of the added bentazone on the inoculum was found. The results of the biodegradability test proper are given in Table B.8.4.3.1-01.

Table B.8.4.3.1-01 Cumulative carbon dioxide evolution after 42 and 117 days during biodegradability of Bentazone after correction for blank values. A, B and C are replicate test bottles

Bentazon (mg/L)		mg CO_2/L	% biodegr.	mg CO_2/L	% biodegr.
10	A	2.8	15.2	10.9	59.7
	B	5.2	27.9	8.9	48.3
	C	3.3	18.1	5.2	27.9
20	A	(-3.3)	-	(-5.6)	-
	B	0.9	2.4	(-0.1)	-
	C	0.7	2.0	0.2	0.5

The results of biodegradability by measurement of loss of parent compound are presented in table B.8.4.3.1-02.

Table B.8.4.3.1-02 Results of the biodegradability of Bentazone

bentazone					
days	0	10		20	
	mg bentazone	mg bentazone	% biodegr	mg bentazone	% biodegr.
0	<0.001	7.8	0.0*	17.3	0.0*
28	<0.001	8.4	0.0	17.0	1.7
117	<0.001	8.1	0.0	19.3	0.0

* by definition

It is not possible to state unequivocally that Bentazone is or is not biodegradable on the basis of these results. Some evidence that biodegradation may occur, in particular in the presence of easily degradable compounds has, however, been obtained.

Comment

The study is considered supplementary as it is neither a guideline study for readily biodegradability, though reference to OECD 301D has been made, nor a water sediment study according to OECD 308.

B.8.4.4 Summary and assessment

Ready biodegradability

A reliable study to test the ready biodegradability of bentazone was not performed. Therefore, the substance has to be considered to be not readily biodegradable.

4.1.2 BOD_5/COD

No data available.

4.1.3 Aquatic simulation tests

No data available.

4.1.4 Other degradability studies

4.1.4.1 Hydrolysis

In the RAR reference is made to chapter B.2.1.15 (IIA 2.9), but there is no study summary presented for Eswein & Panek (1986a). In the section on photolytic degradation, studies are summarized that incubated controls in dark and where no hydrolysis was observed during 8 to 15 days at 20-22 °C.

4.1.4.2 Water, water-sediment and soil degradation data (including simulation studies)

4.1.4.2.1 Water/sediment systems

Overview of the studies from original DAR

Laboratory/field	**conditions**	**Application rate (mg/kg)**	**Duration (d)**	**Reference**
laboratory	aerobic/dark/25°C	9.8	374	Gerhardt and Hamm,

				1987,WAS95-00110
laboratory	aerobic/dark/20°C	1.0	100	Bieber 1994, WAS95-00111
Field	California	2x1.12	360	Evans, 1994, WAS95-00112
Field	Mississippi	2x1.12	360	Evans, 1994, WAS95-00112
Field	Louisiana	0.6/0.6/0.4	103	Ross et al,1989, WAS95-00113
laboratory	anaerobic/dark/25°C	9.8	365	Gerhardt and Hamm, 1987, WAS95-00114

reference	:	Gerhardt and Hamm, WAS95-00110	GLP statement	:	no
Type of study	:	Water sediment	Guideline	:	EPA Guideline § 162-4
Year of execution	:	1987	Acceptability	:	additional
test substance	:	^{14}C phenyl ring labeled bentazone	Previous evaluation	:	In original DAR, acceptable

Method

An aerobic aquatic metabolism study with ^{14}C phenyl ring labelled bentazone was performed at 25°C using a clay soil and a sandy loam soil and natural water from a deep well (Greenville, Mississippi). Prior to start of the experiments the microorganisms in the soils were activated for 14 days. Ten g of activated soil and 90 ml of deep well water were filled in duplicate into an Erlenmeyer flask. Finally the flasks were closed with sterile cotton plugs and shaken (120 rpm) in the dark. Duplicates were run with both soil types at spiking rates of 9.8 mg/kg. Samples were taken 0, 15, 30, 60, 90, 210, 374 and 374 days of incubation including a sterile control experiment. The soil-water suspensions were centrifuged and the resulting soil and aqueous samples were worked up separately. The soil was extracted with methanol, the extracts were methylated with diazomethane and the residues redissolved were subjected to preparative thin layer chromatography analysis and the samples finally analyzed by GC/MS. After methanol extraction the soil was furhter analyzed by extracting with sodium pyrophosphate followed by an extraction with NaOH. Finally the distribution of radioactivity was determined by LSC resp. combustion in a sample oxidizer. The aqueous phase was extracted with ethyl acetate after acidification and the extracts were analyzed by radio TLC and further

worked up like the methanol extracts (see above). During the experiments the mineralization rate was determined by trapping carbon dioxide in an alkaline scintillation cocktail and by measuring the radioactivity by LSC.

Guidelines: The investigation was conducted related to EPA Guideline § 162-4.

Characterisation of test system:

Table B.8.4.3.2-02 water sediment charateristics

system	**Clay [%]**	**Silt [%]**	**Sand [%]**	**Org.C [%]**	**CEC [meq/100g]**	**pH water**	**Redox water [mV]**	**DOC water {mg/L]**
Sandy loam	11.9	16.1	72	0.5	10.6	6.1	-	-
clay	33.7	24.8	41.5	0.7	22.6	6.6	-	-

Results

Recoveries	System 2	System 3
Mass balance (in %)	92.1-99.8	90.5-99.3

The results clearly demonstrate that at each sampling intervall in both sediment types the bulk of radioactivity could be detected in the water phase (see Table B.7.4-2) although with the passage of time the radioactivity becomes more and more adsorbed to the sediment. The amount of extractable radioactivity in the sediments is more or less constant over time. Besides some very small amounts of unpolar fractions bentazone was the main component in the methanol extracts. An amount of 4.2 and 6.3 %, respectively had been incorporated into the organic matter after 90 days. At the end of the study 8.8 respectively 14.2 % had been non-extractable residues. Thereof 4.6 and 7.3 % respectively could be assigned to the humic acid and fulvic acid fraction of the soil. The main component in the aqueous phase was bentazone, too. Besides this, some very minor amounts of more polar resp. unpolar products could be detected. The mineralization rate was only small, amounting to 0.4 % and 0.8 % after 90 days and 3.1 respectively 4.5 % within 374 days.

Conclusively, microbial degradation and hydrolysis of bentazone evidently play a subordinate role in the aquatic system.

Comment: Acceptable. It is stated that the temperature of 25°C does not comply with the test conditions specified in Annex II and Annex III (20°C). Therefore, the results are taken into account for the assessment (see below) only qualitatively.

RMS comment 2013: The studies were considered acceptable in the original DAR however, based on the information vailable in the original report RMS cannot confirm this study can still be considered acceptable

for quantitative assessment of the degradation in water sediment. The information on the route of degradation is considered acceptable.

reference	:	Bieber, WAS95-00111	GLP statement	:	N
Type of study	:	Water sediment	Guideline	:	BBA guideline IV, 5-1
Year of execution	:	1994	Acceptability	:	Acceptable
test substance	:	^{14}C phenyl ring labelled bentazone	Previous evaluation	:	In original DAR
reference	:	Timme and Frehse, BOD96-000138	GLP statement	:	N
Type of study	:	Water sediment	Guideline	:	BBA guideline IV, 5-1
Year of execution	:	1980	Acceptability	:	Acceptable
test substance	:	^{14}C phenyl ring labelled bentazone	Previous evaluation	:	In original DAR
reference	:	Timme et al, BOD96-00139	GLP statement	:	N
Type of study	:	Water sediment	Guideline	:	BBA guideline IV, 5-1
Year of execution	:	1986	Acceptability	:	**Acceptable**
test substance	:	^{14}C phenyl ring labelled bentazone	Previous evaluation	:	In original DAR

Method

The degradation of the ^{14}C phenyl ring labelled bentazone was investigated in two different water/sediment systems under aerobic conditions over a period of 100 days at 20°C in the dark. 0.34 mg test substance were applied per kg water which is equivalent to an application rate of 1 kg/ha assuming an overspray of a 30 cm deep water source. Each test flask contained a 2.5 cm sediment layer and a 6 cm supernatant water layer. Besides total, volatile and extractable radiocarbon, the metabolic profile was determined at each sampling date. At each sampling date approximately 30 ml water were withdrawn from the system by syringe for determination of 0_2 content, pH and redox potential. Volatile compounds were transferred from the water and the headspace of the flask into the trap by a stream of helium; subsequently the redox potential was determined in the sediment. After separation of the water layer by centrifugation and determination of the radioactivity by LSC, an aliquot was extracted with ethyl acetate after acidification. The extracts were further

investigated by radio-TLC and in some cases by radio-HPLC. Another aliquot was acidified and heated while a stream of nitrogen was passed through the solution. Released $^{14}C0_2$ was trapped in an absorption solution and the radioactivity was determined via LSC. The sediment was extracted with methanol(/water) (ultrasonication at room temperature). After determination of the radioactivity, the solutions were further investigated via radio-TLC and in some cases by radio-HPLC. After drying of the sediment, the radioactivity retained was determined by means of combustion/LSC. The plug of quartz wool impregnated with paraffin oil was extracted with ethyl acetate, and the radioactivity in this solution and the amounts of ^{14}C-labeled carbonate was determined. Finally the samples obtained were investigated via GLC/MS. - Two water/sediment samples per system were heat-sterilize before applicaton of the test substance. The systems were found still sterile at termination of the incubation 100 d after application.

Guidelines: The investigation was conducted according to BBA guideline IV, 5-1.

Characterisation of test system:

Table B.8.4.3.2-03 water sediment charateristics

system	**Clay [%]**	**Silt [%]**	**Sand [%]**	**Org.C [%]**	**CEC [meq/100g]**	**pH water**	**Redox water [mV]**	**DOC water {mg/L]**
Sandy loam	12.3	50.2	37.5	2.1	18	7.5	258	16
sand	0.1	2.8	97.1	0.4	3	6.8	233	11

Results

Recoveries: System 4 System 5

Mass balance (in %) 90.2 - 97.2 87.7 - 96.2

The amount of test substance in the test systems decreased slowly to 62 % or 69 % of applied dose (see table B.7.4-2), respectively at termination, the main portion always being present in the water phase. Consequently DT50 and DT90 could not be observed in the total system and in the water. DT50 in the total systems was calculated (according Timme and Frehse 1980, Timme et al. 1986) to be 523 / 908 days, whereas DT90 was calculated to be > 1000 days for both systems. Besides carbon dioxide, the amount of which comprised 2.6 % in both systems at termination, and some minor components, the only transformation product observed was N-methylbentazone (see diagram B.7.4-1). Its formation was found reversible. After 30 days, its amount went through a maximum of 7 % in the system 4 and 13 % in the system 5. This compound was nearly exclusively found in the water phase. Further degradates in the extract solutions were observed at a low extent only, none of them exceeding 4 % of applied. Residues in the sediment after extraction with organic solvents at termination amounted to approximately 15 % in both systems. These residues were separated into

humins, humic and fulvic acids. As the degradation of the test substance in the sterilized systems was found at a lower extent (absolute difference at termination approximately 2 0 %), it is concluded that in the unsterilized systems microbiological processes essentially contributed to the degradation of bentazone.

Comment: Acceptable.

reference	:	Evans, WAS0=95-00112	GLP statement	:	N
Type of study	:	Water sediment	Guideline	:	EPA 164-1
Year of execution	:	1994	Acceptability	:	Acceptable for special conditions (rice)
test substance	:	Bentazone (not specified)	Previous evaluation	:	In original DAR

Method

This trial was conducted to determine the dissipation, mobility and degradation of bentazone and its metabolites in water and soil following applications of bentazone to rice under normal agriculture practices for rice in the Southeastern United States. Identical tests in Mississippi and Louisiana each included one control plot and two treated plots. Both treatment plots received two applications of the test substance fourteen days apart at 1.12 kg as/A 2.24 kg as/A). For plot 2, the first application was applied to bare ground and then the plot was flooded 24 hours after application. For plot 3, the first application was made to a flooded plot. The second application for both plots 2 and 3 was made to the flooded paddies 14 days after the first application. Water samples were taken at periodic intervals from immediately after flooding until 90 day sample. The plots were dry by approximately 120 days after first application. Periodic soil samples were taken to a depth of 15 cm while the plots were flooded. From the time the paddies were dry (approximately 120 days after the first application) until the end of sampling (360 days), the soil samples were collected to a depth of 122 cm. Water and soil samples were analyzed for bentazone, N-methylbentazone and 8-chlorobentazone. A possible aqueous photolytic degradate was analyzed from selected water samples.

Soil and sediment samples were extracted with methanol/methylene chloride, aliquots of the concentrated extracts were injected onto a C-18 reverse phase HPLC connected to a quadrupole mass spectrometer via a thermospray interface (TSP-LC/MS). Water samples were extracted by passing sample water through a C-18 solid phase extraction (SPE) column and eluting with methanol. Aliquots of the concentrated extracts were further analyzed as it is described for the soil samples. For analysis of the photolytic degradate water

samples were filtered through a strong anion exchange solid phase extraction cartridge and eluted with HC1. Aliquots were injected onto a C-18 reverse phase HPLC and analyzed on a UV detector.

Guidelines: This study was conducted to satisfy the US Pesticide Assessment Guideline, Subdivision N, Environmental Fate, Series 164-2, Aquatic Field Dissipation.

Mississippi:

Application: 1st applications to dry/flooded paddy:July 14, 1992

2nd applications to flooded paddies :July 28, 1992

Characterisation of test systems: Soil(sediment) : Clay, clay. See table B.8.4.3.2-04.

Weather conditions : See table B.8.4.3.2-05

.Louisiana:

Application: 1st applications to flooded paddies:July 8, 1992

2nd applications to flooded paddies:July 28, 1992

Characterisation of test systems: Soil(sediment) : Sandy loam (0-30cm), Sandy loam (0-46 cm)

Table B.8.4.3.2-04 water sediment charateristics

system	**Clay [%]**	**Silt [%]**	**Sand [%]**	**Org.C [%]**	**CEC [meq/100g]**	**pH water**	**Redox water [mV]**	**DOC water [mg/L]**
Sandy loam	7.7	38.3	54	0.9	5.8	5.3	-	400
Sandy loam	6.7	37	56.3	0.7	5.5	5.1	-	364

Table B. 8.4.3.2-05: Weather conditions/ Aquatic field dissipation studies of bentazone in Mississippi and Lousiana

DATE		AIR TEMP (average)	SOIL TEMP (average)		PRECI
YEAR	MONTH	(°C)	(°C)		(mm)
Mississippi			*5 cm*	*15 cm*	
1992	Jul	25.8	28.1	28.1	117
	Aug	22.7	26.2	26.4	**93**
	Sept	21.0	24.0	24.3	44
	Oct	16.6	18.5	18.7	**25**
	Nov	9.2	11.9	12.4	124

	Dec	**6.8**	8.1	**8.3**	92
1993	Jan	**5.3**	**7.1**	**7.4**	**87**
	Feb	6.1	**7.1**	**7.4**	54
	Mar*	10.5	10.4	**8.4**	100
	Apr**	14.8	15.3	16.8	161
	May	19.9	21.6	21.6	104
	Jun	25.0	25.9	25.8	62

* There were equipment problems from March 17 to March 31. Only the 2 in soil temperatures were averaged from a full month's data

** There were equipment problems from April 1 to April 21. Only the 2 in soil temperatures were averaged from a full month's data

Table B. 8.4.3.2-05: (continued)

	DATE	**AIR TEMP (average)**	**SOIL TEMP (average)**		**PRECI**
YEAR	**MONTH**	**(°C)**	**(°C)**		**(mm)**
Louisiana			*5 cm*	*15 cm*	
1992	Jul	25.6	28.7	28.5	131
	Aug	26.0	29.1	29.1	149
	Sept	26.8	29.7	29.7	**70**
	Oct*	21.3	23.9	24.3	**46**
	Nov **	-	-	-	-
	Dec	11.8	12.3	12.6	**97**
1993	Jan	8.2	11.4	12.0	192
	Feb	10.9	12.1	12.2	**76**
	Mar	14.0	15.1	14.9	119
	Apr	16.8	18.0	18.4	185
	May	21.8	23.6	23.1	198
	Jun	25.9	27.8	27.2	177

* October soil temperatures are from October 1 to October 25

** There was equipment breakdown from October 26 to November 30

Results:

Recovery fortificaction experiment	**Soil**	**Water**
Bentazone	107 +/- 12 %	104 +/- 12 %
N-Methylbentazone	76 +/- 12 %	86 +/- 11 %
9-Chlorobentazone	107 +/- 12 %	100 +/- 18 %

Limits of quantitation: Soil: 10 ug/kg

Water: 1 ug/kg

Water(photodegradate): 4 ug/kg

In order to provide the dissipation of residue in the environmental as a whole, residues in the soil and in the water were combined. Water values were corrected for the daily depth of flood, and both water and soil values were expressed in terms of kg as/ha: All dissipation calculations were performed with the corrected kg as/ha values. These values are refered to as total residue in soil and water. However, discussion of concentration values in regards to the limits of quantitation were done with mg/kg values.

At Mississippi plot 2, the maximum total residue (bentazone and metabolites in soil and water) was 1.26 kg as/ha which was found immediately after the second application. In water, residues dissipated from 0.89 kg as/ha after the second application to less than the limit of quantitation at 76 days after the second application. In soil, the total residues dissipated from 0.370 kg as/ha after the second application to less than the quantitation limit at 346 days after the second application. The half life for total residues in water and soil was 1.6 days.

At Mississippi plot 3, the maximum total residue (bentazone and metabolites in soil and water), 0.87 kg as /ha, occurred immediately after the second application. In water, residues dissipated from 0.77 after the second application to 0.0005 kg as/ha at 76 days following the second application. In soil, residues dissipated from 0.12 kg as/ha to less than the quantitation limit at 76 days after the second application. The half life for total residues in water and soil was 3.8 days.

At Louisiana plot 2, the maximum total residue was 1.49 kg as/ha found immediately after the second application. In water, residues dissipated from 1.19 after the second application to 0.001 kg as/ha at 76 days after the second application. In soil, the total residues dissipated from 0.30 kg/ha to less than the quantitation limit at 345 days after the second application. The half life for total residues in water and soil was 4.9 days.

At Louisiana plot 3, the maximum total residue was 1.29 kg as/ha immediately after the second application. In water, residues dissipated from 1.10 kg as/ha after the second application to less than the limit of quantitation by 76 days after the second application. In soil, the residues dissipated form 0.19 kg as/ha after the second application to less than the quantitation limit by 76 days after the second application. The half life for total residues in water and soil was 3.8 days.

Soil sampling down to 12 0 cm began after the flood dried or approximately 120 days after the first bentazone application. On the clay soil at the Mississippi site, all residues were located in the surface 0-15cm soil samples for both plots 2 and 3. On the sandy loam soil at Louisiana plot 2 where the first application was made to bare ground 24 hours before flooding, residue levels near the

guantitatin limit were found down to the 76 - 91 cm level at 196 days after the second application. In the Louisiana sandy loam soil in plot 3 where both applications were made to a flooded paddy, no residues were found below the 0-15cm level.

The metabolite 8-chlorobentazone was not detected in any water or soil sample. N-methylbentazone was found in some soil samples from both locations of plot 2, N-methylbentazone levels did not exceed 14 ugVkg (limit of quantitation= 10 ug/kg). This metabolite residue was found in the surface 0-15cm or 15-3 0 cm soil samples. The photodegradate was identified in water samples from both locations at a maximum concentration of 8.7ug/kg (limit of guantitation=3.6 ug/kg). Neither N-methylbentazone nor the photodegradate persisted in the environment and meaningful dissipation curves not be obtained for these metabolites.

In summary, bentazone was observed to degrade rapidly in the rice paddy environment. The majority of the bentazone residue in the water was faster degraded than in the sediment. Metabolites were found in negligible amounts and did not persist.

Comment Acceptable - for the special use of bentazone in rice under (comparable) southeuropean conditions.

RMS comment 2013: The study was considered acceptable in the original DAR for special conditions of the use in rice. The current application does not pertain application in rice. The study is just reported for completeness and not used for risk assessment.

reference	:	Gerhardt and Hamm, WAE95-00114	GLP statement	:	N
Type of study	:	Anaerobic water sediment	Guideline	:	162-3, US EPA
Year of execution	:	1987	Acceptability	:	additional
test substance	:	^{14}C phenyl ring labelled bentazone	Previous evaluation	:	In original DAR

Method

An anaerobic aquatic metabolism study employing clay soil and natural water from a deep well (all coming from Greenville, Mississippi) was performed with ^{14}C phenyl ring labelled bentazone, at spiking rate of 9.8 mg/kg. After filling 10 g of activated soil into Erlenmeyer flasks the soil was flooded with 90 ml of deep well water. Subsequently the flasks were hermetically sealed with glas plugs and allowed to stand for 30 days in order to establish oxygen depletion. During this time additionally nitrogen gas was fed into the flasks by slightly evacuating the trials first and adjusting the originated partial vacuum wiht nitrogen gas. This procedure was repeated every second day consecutively in periods of 3x5 min ventilating and 3x5 min adjusting. After this preincubation the sediments were spiked with 4.9 mg as/ml. Finally the trials were put

into a shaking incubator and shaken (12 0 rpm) in the dark at 25° C +/- 1°C until sampling. To get informations whether the breakdown of the compound was caused by microbes or by hydrolysis, sterile trials were performed analogically- but without 30 days preincubation and without nitrogen atmosphere in the trials. 0, 30, 60, 90, 2 62 and 3 65 days after spiking the trials with as, samples were taken and analyzed: The soil-water-suspensions were centrifuged and the resulting soil and aqueous samples were worked up separately. The soil was extracted with methanol and after centrifugation the distribution of radioactivity in both matrices was determined by liquid scintillation counting (LSC) resp. combustion of dried soil samples in a sample oxidizer before detecting the evolved $^{14}C0_2$ via LSC. Aliquots of the evaporated extracts were further analyzed by radio-Thin layer chromatography and aliquots of the concentrated extracts were methylated with diazomethane and the evaporated and redissolved extracts subjected to preparative TLC analysis and after purification and elution analyzed by GC/MS. The remaining soil was further extracted with Na-pyrophosphate and NaOH, acidified and the distribution of radioacivity was determined by LSC resp. combustion in a sample oxidizer. Aliquots of the aqueous phase were taken to determine the radioactivity by LSC. After acidification the samples were extracted with ethyl acetate and the extracts evaporated and analyzed by radioactivity by radio-TLC. The following step (i.e. methylation with diazomethane) were as described for soil (see above). The 60, 182 and 3 65 days trials were bubbled with nitrogen gas to check whether $^{14}C0_2$ can be detected in the gas phase by trapping this substance in a scintillation cocktail and subsequently measuring the radioactivty via liquid scintillation counting.

Guidelines: This study cover the Pesticide Assessment Guidelines, Subdivision N § 162-3, US EPA Washington.

Characterisation of test system, sediments: Sandy loam, sand.

Results

Recoveries : Mass balance (in %): 92.9 - 98.8

At each sampling interval the bulk of radioactivity could be detected in the water phase (see table B.7.4-5). Apart from some very small amounts of polar components, nothing but bentazone could be detected in this phase. Furthermore, with the passage of time the radioactivity becomes more and more adsorbed to the soil matrix yielding in 9.8 % at 365 days after starting the experiment. The amount of extractable radioactivity, apart from day zero, is more or less constant during the course of the experiment and in general less than 6 % of applied. Besides some very small amounts of polar fractions, bentazone was the main component. In ccntrast to the extractable radioactivity the amount of non-extractable radioactivity increased at the first sampling intervals, was almost constant at the second third of the study and accelerated again resulting in 4.3 % of applied at the end of the exmperiment. In the control experiments the timely course of the microbial activity reflects the increasing incorporation of radioactivity into the organic soil matter. The identity of these residues was determined for the 365 days trials: The major part (3.5%) could be assigned to the fulvic acid fraction and one third to the humic acid fracticn. 0.5 % still remained in the soil.

Practically no ^{14}C-mineralization occured under anaerobic conditions.

A half-life estimation could not be performed, because 365 days after spiking with bentazone, still 87.7 % in the water phase and 5.5 % in the sediment could be assignee to bentazone.

Comment: Acceptable. Additional information only

STUDY IIA, 7.8.3/01

Characteristics

reference	:	Matejek B., 2012(a)	Study type	:	Kinetic evaluation of water sediment study
year of execution	:	2009	Incubation time	:	n.a
GLP statement	:	No, GMP	Nominal concentration	:	n.a.
guideline	:	FOCUS (2006)	temperature	:	n.a.
test substance	:	n.a.	DT_{50}		See results
purity	:	n.a	metabolites		See results
			acceptability	:	**acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Method

Kinetic evaluation of the water/sediment study [*Bieber, W.-D. (1994): Degradation of the Test Substance Bentazon in Aerobic Aquatic Environment. - BASF DocID 94/11026*; WAS95-00111] was performed in order to derive persistence and modelling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*

Experimental data of bentazone were analysed at the P-I level (one-compartment approach) for degradation in the whole system as well as dissipation from the water phase and dissipation in the sediment phase of the test systems. At the P-II level (two-compartment approach: water and sediment), the kinetic analysis considered the degradation in water and sediment and the partitioning between both phases.

Metabolite evaluation at level M-I was not possible for any system. In the Krempe test system the identified metabolite N-methyl-bentazone was observed only at very low levels. The metabolite was found in a concentration > 5% AR only once: at day 30. In the Ohlau test system, N-methyl-bentazone reached a

maximum concentration > 10% also at day 30 after application of the test substance. The compound was nearly exclusively found in the water phase with no appreciable detections in the sediment phase.

However, due to the late formation of the metabolite in both test systems only three time points each were available for kinetic fitting and no clear degradation pattern could be identified. Thus, the kinetic assessment of the metabolite did not provide any reliable results.

Overview experimental data used for kinetic modelling

The experimental data used for kinetic analysis are shown in the following tables.

Table B.8.4.3.2-02 Krempe test system: Experimental data of bentazone used for kinetic modelling

Amount of bentazone as [%] of TAR			
Days after treatment	**Whole System**	**Water**	**Sediment[#]**
0d/1	94.1[a]	94.1[b]	-[b]
0d/2	94.6[a]	94.6[b]	-[b]
0.25d/1	89.4	87.7	(1.7)[c]
0.25d/2	87.5	85.8	(1.7)[c]
1d/1	83.3	80.1	(3.2)[c]
1d/2	84.4	81.0	(3.4)[c]
2d/1	86.3	80.9	(5.4)[c]
2d/2	86.0	80.8	(5.2)[c]
7d/1	79.8	71.8	(8.0)[c]
7d/2	81.1	73.7	(7.4)[c]
14d/1	82.7	72.0	(10.7)[c]
14d/2	75.7	65.1	(10.6)[c]
30d/1	73.4	61.7	11.7
30d/2	77.4	65.8	11.6
60d/1	75.1	65.2	9.9
60d/2	72.9	62.6	10.3
100d/1	58.8	50.3	8.5

100d/2	65.1	55.8	9.3

[#] Calculated from study data as difference between whole system and water phase.

[a] The measured value at DAT 0 was set to the mass balance (total recovery).

[b] The measured value at DAT 0 was treated as if the substance was in the water phase.

[c] The data before the maximum occurrence in sediment were not considered for sediment phase modelling.

Table B.8.4.3.2-02 Krempe test system: Experimental data for N-methyl-bentazone used for kinetic modelling

Amount of N-methyl-bentazone [%] as TAR			
Days after treatment	**Whole System**	**Water**	**Sediment[#]**
0d/1	-[a]	-[a]	-[a]
0d/2	-[a]	-[a]	-[a]
0.25d/1	(3.0)[b]	(3.0)[b]	0.0
0.25d/2	(2.4)[b]	(2.4)[b]	0.0
1d/1	(3.2)[b]	(3.2)[b]	0.0
1d/2	(2.7)[b]	(2.7)[b]	0.0
2d/1	(1.8)[b]	(1.8)[b]	0.0
2d/2	(1.5)[b]	(1.3)[b]	0.2
7d/1	(2.1)[b]	(2.1)[b]	0.0
7d/2	(1.9)[b]	(1.9)[b]	0.0
14d/1	(3.4)[b]	(3.4)[b]	0.0
14d/2	(3.1)[b]	(3.1)[b]	0.0
30d/1	8.8	8.8	0.0
30d/2	5.6	5.6	0.0
60d/1	1.0	1.0	0.0
60d/2	1.0	1.0	0.0

Amount of N-methyl-bentazone [%] as TAR			
Days after treatment	**Whole System**	**Water**	**Sediment[#]**
100d/1	4.7	4.5	0.2
100d/2	3.7	3.4	0.3

[#] Calculated from study data as difference between whole system and water phase.

[a] According to *FOCUS (2006)* metabolites which appear at time zero (t0) should be included as parent material. Hence, no t0 samples for the metabolite were considered in the assessment.

[b] The dissipation of the metabolite was evaluated starting at the day of maximum occurrence that was then defined as 0 days after maximum concentration (0 DAMC). All later times were adjusted accordingly as days after maximum concentrations (DAMC).

Table B.8.4.3.2-04 Ohlau test system: Experimental data of bentazone used for kinetic modelling

Amount of bentazone [%] as TAR			
Days after treatment	**Whole System**	**Water**	**Sediment[#]**
0d/1	93.0[a]	93.0[b]	-[b]
0d/2	95.6[a]	95.6[b]	-[b]
0.25d/1	83.5	81.0	(2.5)[c]
0.25d/2	87.4	85.3	(2.1)[c]
1d/1	84.2	78.9	(5.3)[c]
1d/2	83.5	77.4	(6.1)[c]
2d/1	86.9	82.1	(4.8)[c]
2d/2	86.5	79.8	(6.7)[c]
7d/1	87.4	79.3	(8.1)[c]
7d/2	88.0	79.3	(8.7)[c]
14d/1	73.8	63.7	(10.1)[c]
14d/2	74.4	64.1	(10.3)[c]

Amount of bentazone [%] as TAR			
Days after treatment	**Whole System**	**Water**	**Sediment[#]**
30d/1	73.9	63.4	10.5
30d/2	61.7	52.2	9.5
60d/1	77.8	70.6	7.2
60d/2	80.5	71.7	8.8
100d/1	66.9	59.8	7.1
100d/2	70.1	62.0	8.1

[#] Calculated from study data as difference between whole system and water phase.

[a] The measured value at DAT 0 was set to the mass balance (total recovery).

[b] The measured value at DAT 0 was treated as if the substance was in the water phase.

[c] The data before the maximum occurrence in sediment were not considered for sediment phase modelling.

Table B.8.4.3.2-05 Ohlau test system: Experimental data for N-methyl-bentazone used for kinetic modelling

Amount of N-methyl-bentazone [%] as TAR			
Days after treatment	**Whole System**	**Water**	**Sediment[#]**
0d/1	-[a]	-[a]	-[a]
0d/2	-[a]	-[a]	-[a]
0.25d/1	(2.2)[b]	2.2	0.0
0.25d/2	(2.4)[b]	2.3	0.1
1d/1	(2.2)[b]	2.0	0.2
1d/2	(1.8)[b]	1.8	0.0

Amount of N-methyl-bentazone [%] as TAR			
Days after treatment	**Whole System**	**Water**	**Sediment[#]**
2d/1	(1.3)[b]	1.0	0.3
2d/2	(0.4)[b]	(0.1)[b,c]	0.3
7d/1	(0.1)[b,c]	(0.1)[b,c]	0.0
7d/2	(0.1)[b,c]	(0.1)[b,c]	0.0
14d/1	(4.5)[b]	4.5	0.0
14d/2	(4.0)[b]	4.0	0.0
30d/1	10.0	10.0	0.0
30d/2	15.1	15.1	0.0
60d/1	1.6	1.6	0.0
60d/2	1.0	1.0	0.0
100d/1	2.4	2.4	0.0
100d/2	1.6	1.6	0.0

[#] Calculated from study data as difference between whole system and water phase.

[a] According to FOCUS (2006) metabolites which appear at time zero (t0) should be included as parent material. Hence, no t0 samples for the metabolite were considered in the assessment.

[b] The dissipation of the metabolite was evaluated starting at the day of maximum occurrence that was then defined as 0 days after maximum concentration (0 DAMC). All later times were adjusted accordingly as days after maximum concentrations (DAMC).

[c] Values <0.1 were set to 0.1.

Data were analysed using ModelMaker 3.0.4 The χ^2 - error levels and the reliability based on t-test were calculated using the Excel sheet FOCUS_DEGKIN_v2 provided by the FOCUS workgroup.

Results

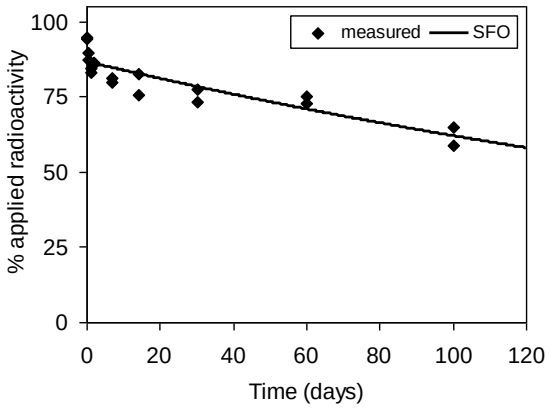
The initial fit for all compartments (total system, water, sediment) was performed using SFO kinetics. In a further step, it was tested whether a bi-phasic FOMC kinetic model was more appropriate, and if so, DFOP and HS kinetics were implemented. Graphical presentations of the tested kinetic models and the results of the χ^2 - test and all other statistical endpoints used in the decision-making process are given further below.

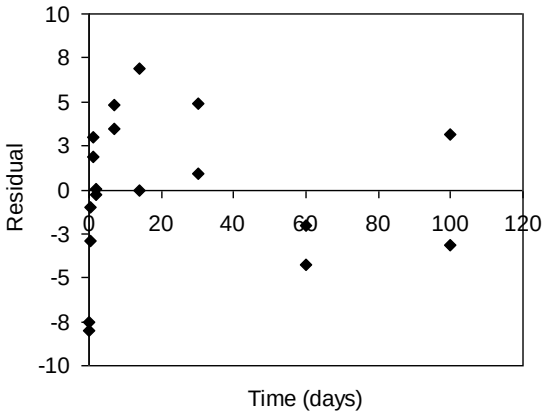
The evaluation of the results of the Krempe test system at P-I level showed that degradation in the total system was best described by SFO kinetics. Dissipation from the water phase was best described by the DFOP kinetic model, whereas dissipation from the sediment was best described by SFO kinetics. The evaluation of the results of the Ohlau test system at P-I level showed that degradation in the total system and dissipation from the water and sediment phase could be adequately described by SFO kinetics.

The selected best-fit models are presented graphically in Figure B.8.4.3-01 and fig B.8.4.3-02, and a summary of the corresponding model parameters (including reliability based on t-test) is reported in Table B.8.4.3-05

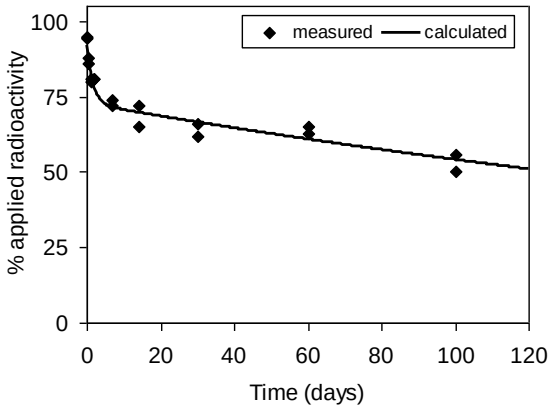
Figure B.8.4.3.2-01 Level P-I, bentazone: best-fit kinetic models for total system, water and sediment (Krempe system)

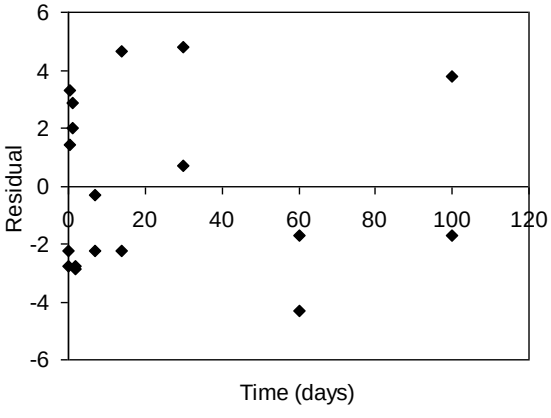
Total System – SFO kinetics



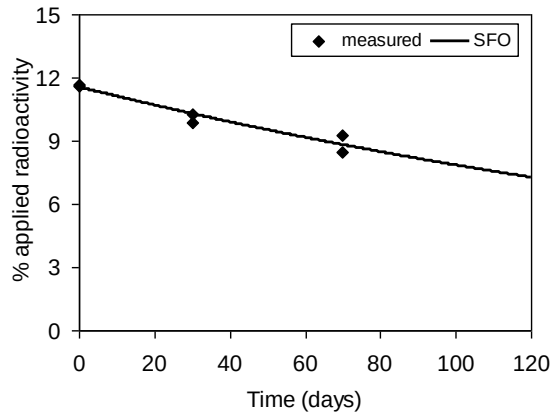


Water phase – DFOP kinetics





Sediment phase – SFO kinetics



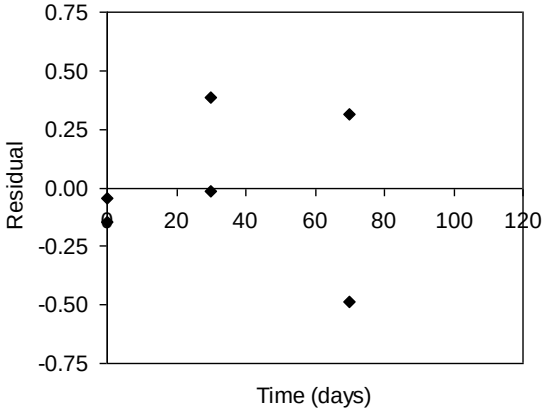
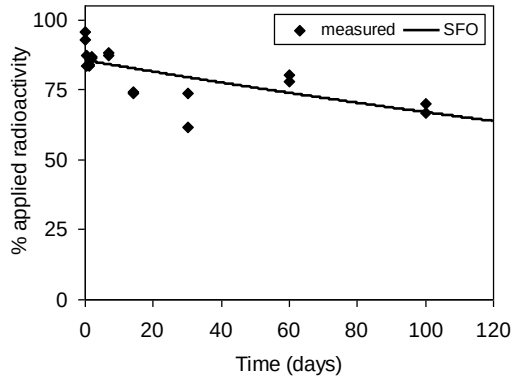
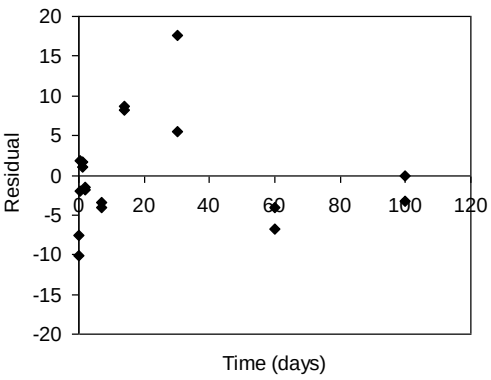


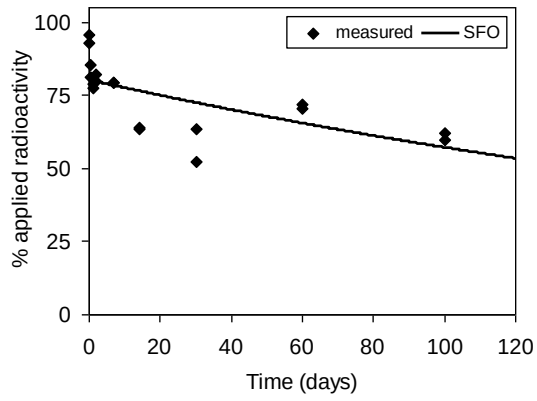
Figure B.8.4.3.2-02 Level P-I, bentazone: best-fit kinetic models for total system, water and sediment (Ohlau system)

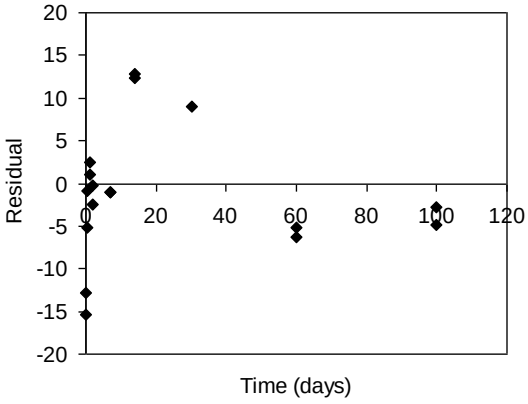
Total System – SFO kinetics





Water phase – DFOP kinetics





Sediment phase – SFO kinetics

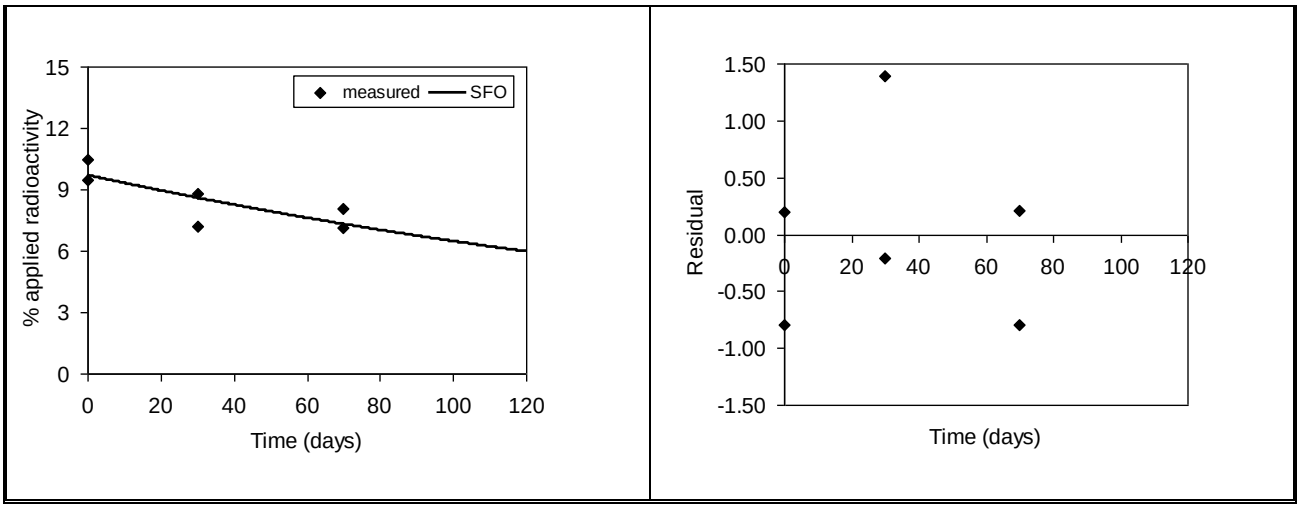


Table B.8.4.3.2-05 Level P-I, bentazone: Estimated parameters for best-fit kinetic models

System*	Compartment **	Kinetic model	Parameter	Estimated value	Standard error	χ^2	Type I error rate	DT_{50} [d]	DT_{90} [d]
Krempe	Total system	SFO	M0 [%TAR]	86.593	1.281	2.6	-	207	688
			k [d^{-1}]	0.003	< 0.001		< 0.05		
	Water**	DFOP	M0 [%TAR]	91.845	1.879	3.4	-	156	701
			k1 [d^{-1}]	0.600	0.200		< 0.05		
			k2 [d^{-1}]	0.003	0.001		< 0.05		
			g [-]	0.208	0.025		< 0.05		
	Sediment**	SFO	M0 [%TAR]	11.555	0.230	0.7	-	179	595
			k [d^{-1}]	0.004	0.001		< 0.05		

System*	Compartment **	Kinetic model	Parameter	Estimated value	Standard error	χ2	Type I error rate	DT_{50} [d]	DT_{90} [d]
Ohlau	Total system	SFO	M0 [%TAR]	85.423	2.054	4.4	-	283	940
			k [d^{-1}]	0.002	< 0.001		< 0.05		
	Water**	SFO	M0 [%TAR]	80.172	2.749	6.6	-	204	678
			k [d^{-1}]	0.003	< 0.00		< 0.05		
	Sediment**	SFO	M0 [%TAR]	9.701	0.588	2.8	-	171	569
			k [d^{-1}]	0.004	0.002		< 0.05		

* $DegT_{50}$

** $DisT_{50}$

Summary of level P-II kinetic evaluation

Degradation of bentazone in water and sediment as well as partitioning between both phases was analysed according to the P-II level kinetic concept (two-compartment approach). of the FOCUS guidance document. A compartment model was used and SFO kinetics were considered for the transfer and degradation rates.

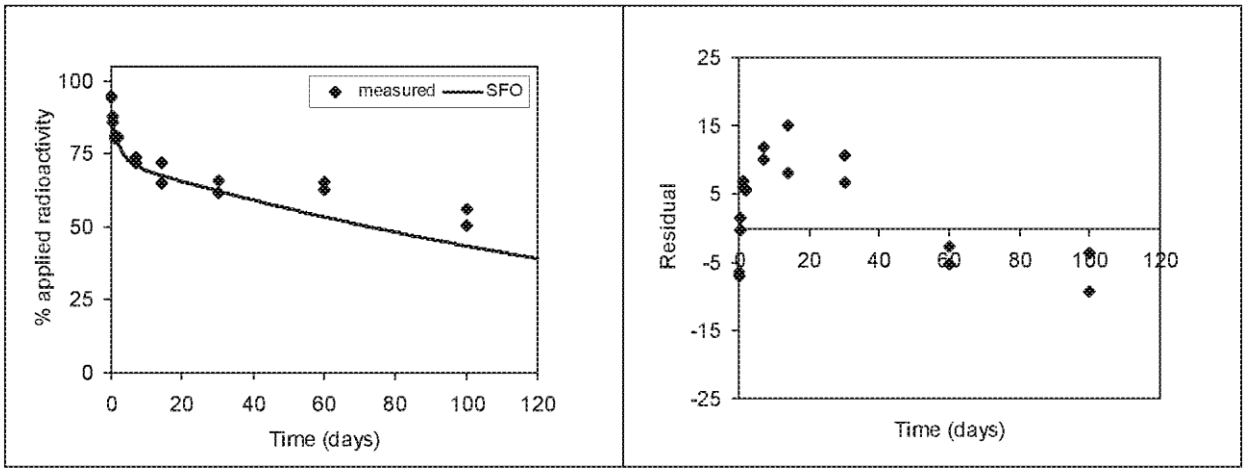
It must be shown that the fit is visually and statistically acceptable, i.e. the results must be consistent with environmental fate data, degradation rates in both compartments must be significantly greater than zero as shown by t-test and the F_{sed} check must be passed.

P-II level analysis was performed for both systems but resulted in a weak fit to the measured data. For the Krempe system water phase, a visually acceptable fit could not be obtained, because the selected compartment model overestimated the degradation in the water phase. Also for the sediment phase, a visually acceptable fit could not be obtained. For the Ohlau system a visually acceptable fit could not be obtained for either compartment. Furthermore, t-test and F_{sed} check failed for both systems and thus kinetic evaluation at P-II level was not pursued further.

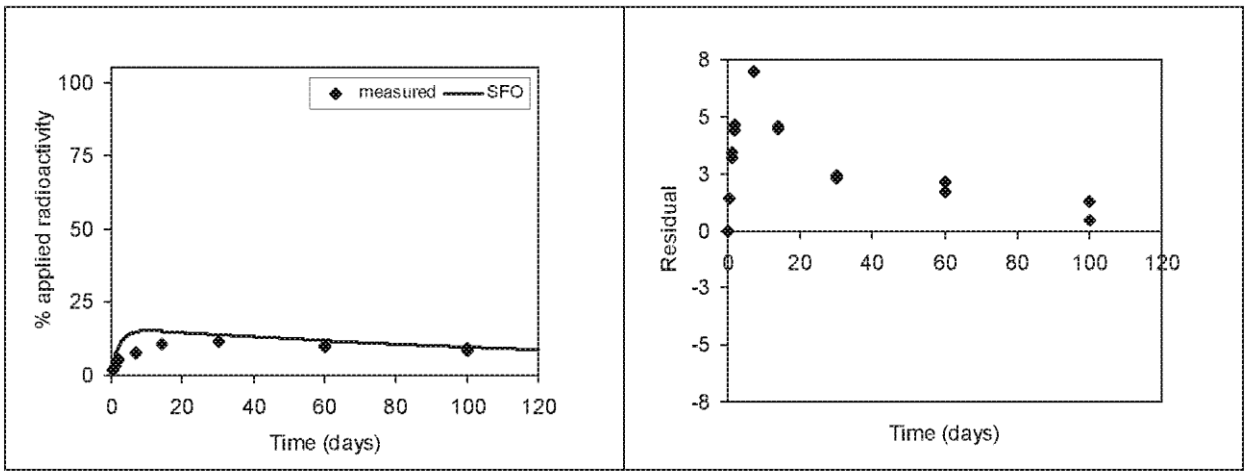
All graphical presentations of the kinetic models and the results of the χ^2 - test and other statistical endpoints are shown below.

Figure B.8.4.3.2-02 Level P-II, kinetic fitting , water and sediment (Krempe system)

Water phase - SFO



Sediment phase - SFO

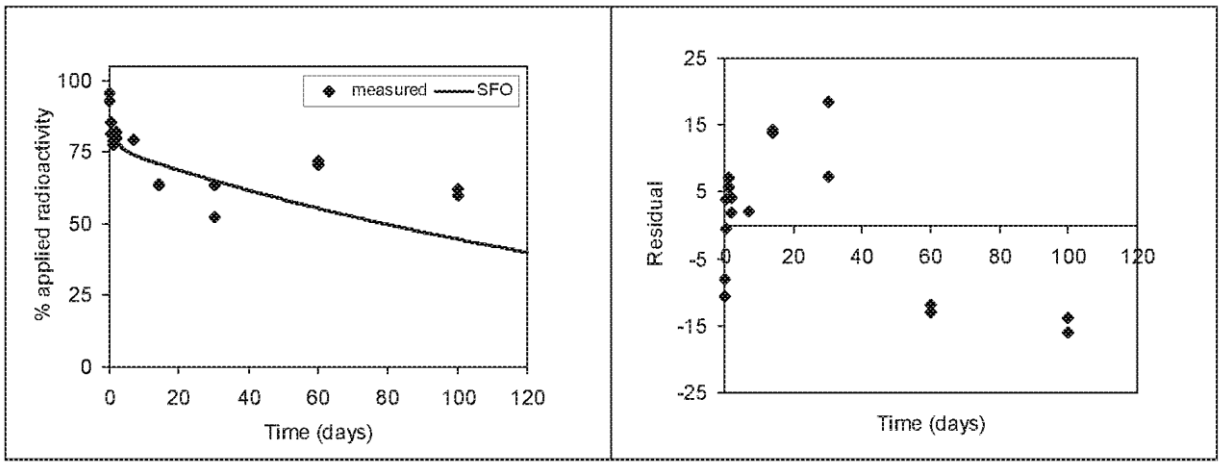


Compartment	Goodness of fit (r^2)	Visual	Chi² err. (%)	DegT50 / DegT90
Water	0.719	poor	6.1	109.8 / 364.7
Sediment		poor	29.9	>1000 / >1000

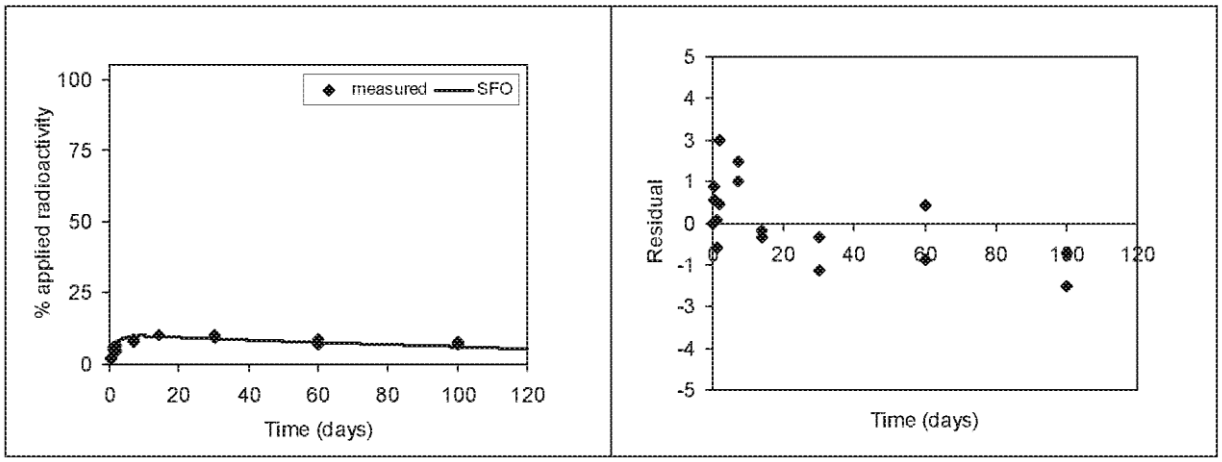
Parameter	Initial	Optimised ± Err	t-test
M0	94.4	87.530± 2.980	not required
Msed	1.7	Fix Msed	not required
k_ws	0.05	0.006±0.055	not significant
k_ss	0.05	5.000E-09±0.583	not significant
r_ws	0.5	0.075±0.074	not significant
r_sw	0.5	0.340±1.088	not significant

Figure B.8.4.3-02 Level P-II, kinetic fitting , water and sediment (Ohlau system)

Water phase - SFO



Sediment phase - SFO



Compartment	Goodness of fit (r^2)	Visual	Chi² err. (%)	DegT50 / DegT90
Water	0.345	poor	8.0	112.5 / 373.8
Sediment		poor	8.4	>1000 / >1000

Parameter	Initial	Optimised ± Err	t-test
M0	94.3	85.013±4.258	not required
Msed	2.3	Fix Msed	not required
k_ws	0.005	0.006±0.088	not significant
k_ss	0.005	5.000E-09±0.580	not significant
r_ws	0.5	0.057±0.121	not significant
r_sw	0.5	0.412±1.085	not significant

RMS Comment

The results of the kinetic evaluation as provided are acceptable. The level P-I results are used for risk assessment.

Second notifier data

STUDY IIA, 7.8.3/02

Characteristics

reference	:	De Vries (1996)	Study type	:	water sediment study
year of execution	:	1996	Incubation time	:	106 d
GLP statement	:	yes	Nominal concentration	:	0.48 mg/L
guideline	:	No internationally accepted	temperature	:	Not reported
test substance	:	^{14}C Bentazone. Batchno not provided	DT_{50}		See results
purity	:	Radiochemical purity 99.5 - 100%	metabolites		See results
			acceptability	:	**acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Method

This study is a confirmatory study of the data available in the original DAR. A water sediment study on 2 systems was performed, Oostvaardersplassen and Schoonrewoerdse Wiel. On each location, about 100 liter of water and 30 kg of sediment, taken from the top layer, were collected and transported to Notox. The next day, approximately 20 Liter of the water fraction was filtered over a 180 µm

sieve; the sediment was sieved over a 2 mm sieve. 22 bottles were filled with appropriate amounts of water and sediment, and were stored under experimental conditions for a period of 8 weeks in order to reach equilibrium.

To determine the degradation rate of bentazon flasks were filled with wet sediment (2-2.5 cm) and an amount of water. Water was gently stirred from the top. The system was ventilated with $C0_2$ free moistened air at a flowrate of about 15-30 ml/min. The outcoming air was passed through a trapping system of 2N NaOH (50 ml, 2 bottles in series) for trapping of $C0_2$, and one bottle of 2- methoxyethanol (50 ml) for

trapping of organic volatiles. The water level of the water/sediment samples was checked (by weight) and adjusted if necessary. Solutions of ^{14}C-labelled bentazon were prepared in methanol. An amount of the labelled substance was added to a flask filled with methanol. The specific activity was determined by triplicate measurements (LSC) of an aliquot. Non-labelled bentazon and the metabolites anthranilic acid and anthranilic acid-iso-propylamide were also prepared and diluted in methanol.

The light/dark regime was 12 hrs light (500-8000 Lux) (during icubation). Because of algae growth in the bottles, the light/dark regime was reduced to 8 hrs light and 300-350 Lux until the end of the test.

The characterisation of the water/sediment system is reported in table B.8.4.3.2-06.

Table B.8.4.3.2-06 water/sediment charaterisation

Parameter	Oostvaardersplassen (OVP)	Schoonrewoerdse Wiel (SW)
Water level[d]	20 to 30 cm	1.5 m
Temperature (°C)[d]	not registered	22.5
pH[d]	8.0	9.0
Oxygen content (mg/l)[d]	10.8	15.2
Water redox potential (mV)[d]		
10 cm below water surface	-200	-203
10 cm above soil surface)	-225	not registered
Sediment redox potential	-225	-230
Sediment cation exchange capacity[b] (CEC, mEq/100 g soil)	27.1	30.7
Sediment dry matter (%)[a]	27	18
Organic carbon content (OC)		
Sediment (total, TOC,%)	5.7	14.6
Water[b] (dissolved, DOC, mg/l)	23.5	23.5
Total hardness water[b] (mg/l $CaCO_3$)	164	163
Phosphorous (total)[b]		
Sediment (mg/kg)	849	1545
Water (mg/l)	0.1	0.1
Nitrogen (total)[b]		
Sediment (mg/kg)	3.9	0.7
Water (mg/l)	7.2	11.2
Particle size distribution (%)		
< 2 µm (clay)	20.7	15.1
2-6 µm (silt)	4.5	8.9
60-20 µm (silt)	0.8	29.9
20-63 µm (silt)	30.3	25.2
63-2000 µmc (sand)	43.7	20.9
ATP sediment[c] (µg/kg dry weight)		
beginning	75	12
end	95	149

a After decanting the water layer and drying for 16 hours at 105°C.
b Determined under GLP at "soil survey & land research centre", Cranfield University, Silsoe, Bedford, MK45 4DT
c Non-GLP determination at EPAS, Eco Process Assistance, IIC-University Gent, Technologiepark 3 - 9052 Gent (Zwijnaarde)
d Determined just before sampling

After a pre-incubation period of 8 weeks radiolabelled bentazon was added to the water/sediment samples at a concentration of 0.48 mg/1 water, corresponding to the maximum recommended field rate of bentazon of 1.44 kg a.i./ha. Only labelled substance was used to spike the water/sediment sample,s spiking solvent was methanol.. Parallel with the radiolabelled samples, 2 blank samples were spiked with unlabelled bentazon in methanol. Samples were taken at T= 0, 8, 14, 28, 35, 42, 71, and 106 days (2 bottles per sampling date). The water was decanted from the sediment and both matrices were stored refrigerated or deep frozen

until the day of extraction.

Determination of radioactive volatiles

On each sampling date the activity in the 2-methoxyethanol trap and the NaOH traps was analysed by counting an aliquot by LSC. The activity was quantified and expressed as percentage of applied (mass balance).

Determination of the radioactivity in the water fraction

The decanted water fraction was transferred to a separatory funnel and acidified using about 3 ml of 1N HCl (pH was then approximately 2.5). Saturated sodium chloride solution (100 ml) was added and the water was extracted three times for 1 minute with 100 ml dichloromethane. The combined dichloromethane fractions were evaporated until dryness and dissolved in 5 ml methanol. An aliquot was diluted with cocktail, and the activity was measured and quantified using LSC. Another aliquot was used for identification with radio-TLC

Determination of extractable residues in the sediment

Each sediment sample was acidified with 1 ml of 1N HCl and was extracted three times for 1 hr (shaking at 200 rpm) with 100 ml acetone. The supernatant was vacuum filtered on a Buchner filter with filter paper into a roundbottom flask. The filtrates were combined and the acetone was evaporated using a rotary evaporator. After addition of 25 ml of saturated sodium chloride and 1 ml of 1N HCl, the aqueous residue was extracted three times for 1 minute with 50 ml dichloromethane. The combined dichloromethane fractions were evaporated to dryness using a rotary evaporator and were redissolved in 5.0 ml methanol. The activity in the methanol extract was quantified by counting 100 µl of the resulting solution in 5 ml cocktail on LSC. The total activity in the water fraction was calculated and expressed as percentage of applied (mass balance). Identification was done with radio-TLC

Determination of non-extractable ^{14}C-residues in the sediment

After the extraction procedure of the sediment, at least three 1 gram subsamples were combusted using an oxidizer. The evolving ^{14}C-$C0_2$ was trapped and, quantified on LSC and expressed as percentage of applied (mass balance).

Determination of bentazon and metabolites in sample extracts

For identification of the components in the water and the sediment extracts, differences in retention behavior on TLC were used.

Results

Material balance

Table B.8.4.3.2-07 Oostvaardersplassen. Material balance of radio-activity *(%* recovery) in the water/sediment samples.

Time [days]	Total $^{14}CO_2$	Water fraction	sediment fraction	Bound residues	Recovery (total)
0		96.27	6.17	0.5	102.9
0		88.82	3.36	0.5	92.7
8	0.02	74.75	23.61	3.2	101.6
8	0.02	65.09	22.93	2.9	90.9
14	0.03	45.79	10.02	0.9	56.7[2]
14	0.02	47.12	9.64	0.9	57.7[2]
28	0.14	71.31	26.36	6.2	104.0
28	0.16	73.23	25.43	5.0	103.8
35	0.10	73.79	22.82	4.9	101.6
35	0.14	68.80	24.79	5.6	99.3
42	0.19	73.11	22.71	4.2	100.2
42	0.15	77.64	23.63	2.9	103.3
71	0.80	75.19	28.93	2.8	107.7
71	0.72	74.04	25.83	7.0	107.6
106	0.74	66.02	25.95	6.3	95.9
106	0.53	67.66	27.48	5.1	100.8

1 Volatile organics were in all cases determined to be <0.01%
2 Mass balance is too low; all t=14 results are therefore omitted in the discussion

Table B.8.4.3.2-08 Schoonrewoerdse wiel Material balance of radio-activity *(%* recovery) in the water/sediment samples.

Time [days]	Total $^{14}CO_2$	Water fraction	Sediment fraction	Bound residues	Recovery (total)
0		98.46	6.47	0.3	105.2
0		87.73	1.94	0.6	90.3
8	0.03	60.67	35.15	4.4	100.3
8	0.02	55.99	30.36	4.2	90.8
14	0.04	51.19	12.15	1.5	64.9[3]
14	0.05	52.16	21.59	2.0	75.8[3]
28	0.16	65.06	25.79	6.2	97.2
28	0.12	67.32	25.80	5.2	98.4
35	0.11	0[2]	26.24	5.8	-[2]
35	0.07	0[2]	29.63	6.3	-[2]
42	0.20	65.72	27.96	5.3	99.2
42	0.13	66.91	23.12	6.0	96.2
71	1.14	50.51	48.67	2.6	102.9
71	0.5	50.50	35.11	6.7	92.8
106	1.34	53.47	38.42	10.5	103.7
106	0.74	52.3	35.71	7.6	96.4

1 Volatile organics were in all cases determined to be <0.01%.
2 Extraction of the water sample went wrong. Total value cannot be calculated
3 Mass balance is too low; all t=14 results are therefore omitted in the discussion

Identification and quantification of metabolites

Table B.8.4.3.2-09 Oostvaardersplassen. Identification (reversed phase and straight phase TLC) and quantification of the components in water and sediment (% of applied * C)

Time (days)	WATER[1]					SEDIMENT[1]				
	Total	RP-TLC bentaz.	RP-TLC metab.	SP-TLC bentaz.	SP-TLC metab.	Total	RP-TLC bentaz.	RP-TLC metab.	SP-TLC bentaz.	SP-TLC metab.
0	96.27	96.27		96.27		6.17	5.35	0.82	5.32	0.85
0	88.82	88.82		88.82		3.36	1.34	2.02	1.50	1.86
8	74.75	73.02	1.73	73.05	1.70	23.61	23.61		23.61	
8	65.09	65.09		65.09		22.93	22.93		22.93	
14	45.79	45.79		45.79		10.02	9.78	0.24	9.70	0.32
14	47.12	47.12		47.12		9.64	9.64		9.43	0.21
28	71.31	71.31		71.31		26.36	26.36		25.50	0.86
28	73.23	73.23		73.23		25.43	24.42	1.01	24.48	0.95
35	73.79	68.23	5.56	73.79		22.82	22.82		20.71	2.11
35	68.8	68.80		67.46	1.34	24.79	24.79		22.63	2.16
42	73.11	73.11		73.11		22.71	21.48	1.23	21.59	1.12
42	77.64	77.64		77.64		23.63	22.31	1.32	19.46	1.47
71	75.19	75.19		69.70	5.49	28.93	27.50	1.43	28.23	0.70
71	74.04	74.04		68.75	5.29	25.83	24.71	1.12	25.31	0.52
106	66.02	66.02		66.02		25.95	21.92	4.03	22.54	0.26
106	67.66	67.66		65.94	1.72	27.48	27.48		26.24	1.24

[1] Metabolite identified with SP-TLC as anthranilic acid; identity not confirmed with RP-TLC

Table B.8.4.3.2-10 Schoonrewoerdse wiel. Identification (reversed phase and straight phase TLC) and quantification of the components in water and sediment (% of applied * C)

Time (days)	WATER					SEDIMENT[1]				
	Total	RP-TLC bentaz.	RP-TLC metab.	SP-TLC bentaz.	SP-TLC metab.	Total	RP-TLC bentaz.	RP-TLC metab.	SP-TLC bentaz.	SP-TLC metab.
0	98.46	98.46		98.46		6.47	1.71	4.76	1.19	5.28
0	87.73	87.73		87.73		1.97	0.98	0.99	1.02	0.95
8	60.67	60.67		60.67		35.15	35.15		35.15	
8	55.99	55.99		52.47	3.52[2]	30.63	29.78	0.85	30.11	0.52
14	51.19	51.19		51.19		12.15	11.65	0.50	11.78	0.37
14	52.16	52.16		52.16		21.59	20.64	0.95	21.59	
28	65.06	65.06		65.06		25.79	24.13	1.66	25.79	
28	67.32	67.32		67.32		25.80	25.30	0.5	25.80	
35	-	-		-		26.24	25.16	1.08	25.06	1.18
35	-	-		-		29.63	28.71	0.92	28.66	0.97
42	65.72	65.72		65.72		27.96	24.31	3.65	25.55	2.41
42	66.91	66.91		66.91		23.12	20.85	2.27	20.64	2.48
71	50.51	50.51		50.51		48.67	45.68	2.99	48.67	
71	50.50	50.50		50.50		35.11	31.29	3.82	33.88	1.23
106	53.47	53.47		53.47		38.42	36.02	2.40	37.49	0.85
106	52.30	52.30		52.30		35.71	32.36	3.35	34.39	1.32

[1] Metabolite identified with SP-TLC as anthranilic acid; identity not confirmed with RP-TLC
[2] Not identified

Water and sediment extracts of T=106 days samples were analyzed by ionspray-MS/MS in order to confirm the absence of metabolites. The MS results did not indicate the presence of significant amounts of hydroxybentazone, anthranilic acid-iso-propylamide or anthranilic acid.

For Oostvaardersplassen, the overall ^{14}C-recovery varied between 91% and 108%. For Schoonrewoerdse Wiel, the overall ^{14}C-recovery varied between 90% and 105%. The target limits for the mass balance were

met. The $^{14}C02$ concentration in the traps was very low and achieved a maximum concentration of 1.34% of the applied radio-activity. The volatile organics in the traps were in all cases determined to be negligible (<0.01%). The amount of non-extractables that was recovered by combustion of the sediment after extraction varied between 0.3% and 10.5% with no significant increase or decrease with time. The metabolite concentrations were below 5.5%.

The bentazon concentration in the water/sediment systems achieved an equilibrium after 8 days and this equilibrium remained until the last sampling date (T=106 days). Between 8 days and 106 days after application, the bentazon concentrations in the water were in the range 50%-75%; in the sediment 19%-49%. No decline of the bentazon concentration was observed in this time period. As no decline of the bentazon concentration was observed in the water/sediment systems between 8 days and 106 days after application, neither the DT50 nor the DT90 could be calculated.

RMS Comment

The study was performed for a Dutch registration and did not follow any recognised international guideline. At the time of the study such guidelines did not exist. The study was done under a light dark regime but the influence of the light cannot be determined on the basis of the results. It is stated the study is performed at room temperature but no record is kept of temperature and the real temperature range is not reported. No decline was observed during the study neither degradation nor dissipation. With the methods used no metabolite formation was observed. Analysis for 6-hydroxy bentazone, 8-hydroxy bentazone, anthranilic acid-iso-propylamide and anthranilic acid was performed using TLC. The t=106 days sample was subjected to MS/MS ionspray. No individual metabolite exceeded 5% in water or sediment. The study is not considered to provide quantitative information with regard to the fate and behaviour of bentazon in a water/sediment system under light/dark incubation regime. The study itself does not provide an explanation on the initial decline of bentazone followed by stability for the rest of the study. The study is reliable but does not provide any usefull information for further assessment.

B.8.4.4 Summary and assessment

Aerobic water/sediment studies

Main notifier

In a study with two aquatic systems ("Krempe" and "Ohlau"), most of bentazone remained in the water phase (53 - 61% of the total applied radioactivity (TAR) after 100 days). The maximum observed occurrence of bentazone in the sediment reached a maximum of 12.2% TAR at day 30 after treatment in system "Krempe". It slowly declined in sediment to 9.6% at 100 days.

N-methyl-bentazone was the only major metabolite found at a maximum of 12.5% TAR after 30 days in the water phase. It decreased again to about 2% after 100 days. N-methyl-bentazone is not expected to occur in significant amounts in sediments.

Mineralization was negligible with 2.6% TAR after 100 days. Formation of non-extractable residues was moderate reaching 13.4 - 15.6% TAR at the end of incubation.

Kinetic evaluation of the data revealed reliable level P-I endpoints for the 2 water sediment systems.

Table B.8.4.4-02 Level P-I persistence and modelling endpoints for bentazone

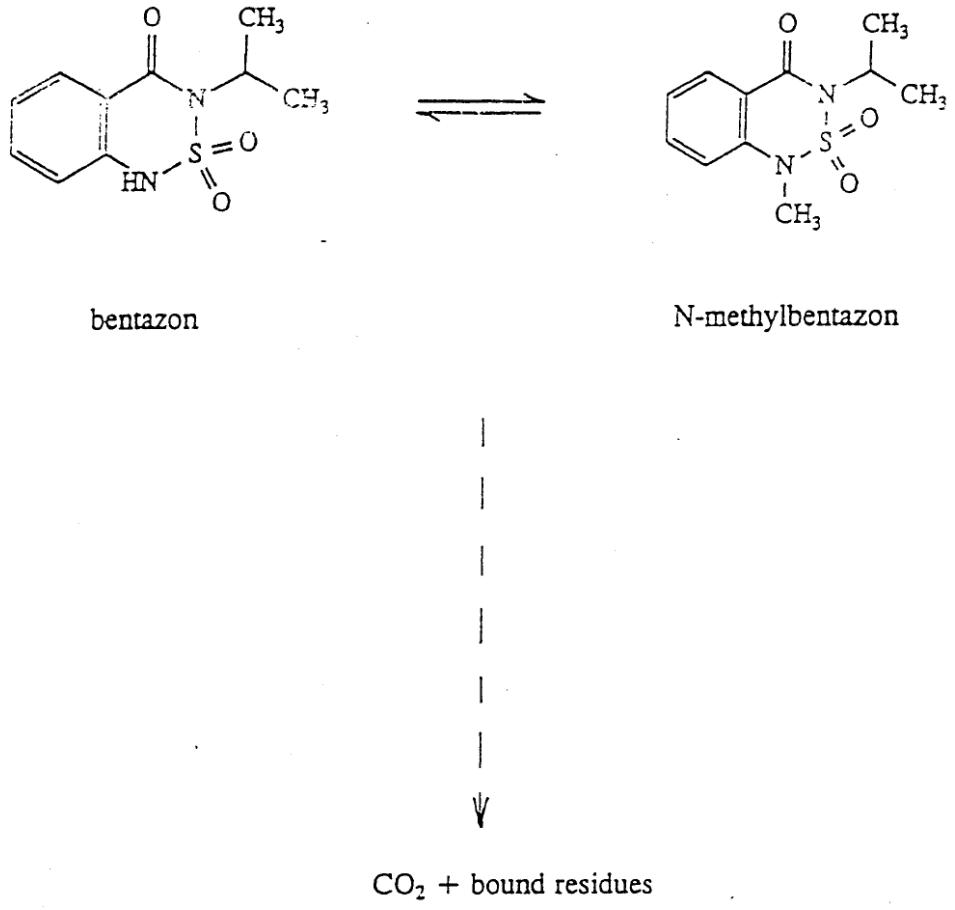
Test System	**Persistence endpoints**			**Modelling endpoints**	
Total System	**Kinetic model**	**$DegT_{50}$ [d]**	**$DegT_{90}$ [d]**	**Kinetic model**	**$DegT_{50}$ [d]**
Krempe	SFO	207	688	SFO	207
Ohlau	SFO	283	940	SFO	283
Geometric mean					**242**
Water phase	**Kinetic model**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Kinetic model**	**$DisT_{50}$ [d]**
Krempe	DFOP	156	701	SFO[#]	235
Ohlau	SFO	204	678	SFO	204
Geometric mean					**219**
Sediment phase	**Kinetic model**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Kinetic model**	**$DisT_{50}$ [d]**
Krempe	SFO	179	595	SFO	179
Ohlau	SFO	171	568	SFO	171
Geometric mean					**175**

[#] according to FOCUS (2006) the half-life from the slow phase of the DFOP bi-phasic model is used as modelling endpoint since 10% of the initially measured concentration was not reached within the experimental period

Second notifier

No additional data on the fate and behaviour of bentazone in water sediment systems were submitted.

Figure 8.4.4-03 Proposed route of degradation of bentazone in water/sediment systems



4.1.4.2.2 Biodegradationin soil

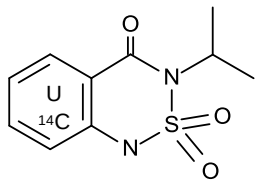
B.8.1 Route and rate of degradation in soil (Annex IIA 7.1.1 and Annex IIIA 9.1.1)

For the current Annex I renewal, a data gap analysis according to new guidelines, new guidance documents and new procedures in kinetic evaluations and exposure assessments was performed, and new studies / evaluations were initiated where considered necessary. In order to provide a comprehensive overview on existing environmental data on bentazone for Annex I renewal, each chapter of this section consists of a summary on the former EU agreed endpoints followed by summaries of the new studies and kinetic evaluations according to the current guidance required in the EU process.

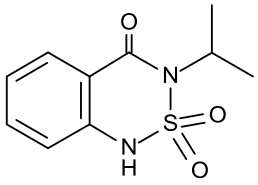
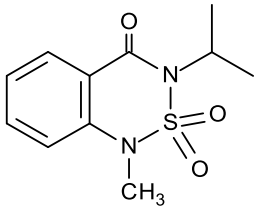
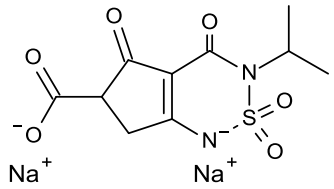
Furthermore, a literature search was performed, and scientific publications were included into the dossier when considered endpoint relevant and being of sufficient high quality. All bentazone related publications on environmental fate found during the literature search were categorized according to (1) being endpoint relevant and of adequate quality; then summaries are provided in the appropriate dossier chapters, (2)

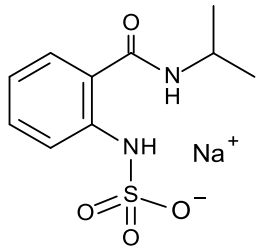
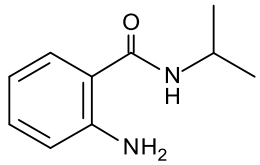
potentially endpoint relevant, but showing no new information or having deficiencies, (3) having no endpoint relevance.

The already peer-reviewed studies as well as the new studies by the main notifier were performed using uniformly phenyl-ring ^{14}C-labelled bentazone. Where different label posistions were used in the original DAR studies this is indicated in the summary.



A concordance list of structures and designations of reference compounds used during environmental fate studies is given below.

Compound designation	**Reference code (Reg. No.)**	**Molecular weight (g/mol)**	**Structure**
Bentazon BAS 351 H M351H000	51929	240.3	
N-methyl-bentazon BH 351-N-me. M351H009	79520	254.3	
"Peak B" M351H023	5819746 (5831080 = disodium-salt)	332.25	

Compound designation	Reference code (Reg. No.)	Molecular weight (g/mol)	Structure
"Peak C" M351H024	268168 (5051517 = sodium-salt)	280.28	
BH 351-AIPAM "AIPAM" M351H025	36848	178.23	

B.8.1.1 Route of degradation in soil (Annex IIA 7.1)

B.8.1.1.1 Aerobic degradation (Annex IIA 7.1.1)

Main notifier data/data from original DAR

reference	:	Drescher and Otto, BOD96-00054.	GLP statement	:	no
Type of study	:	Soil degradation	Guideline	:	unknown
Year of execution	:	1972	Acceptability	:	Not acceptable
test substance	:	^{14}C-bentazone	Previous evaluation	:	In original DAR: acceptable

Method

The route of degradation of ^{14}C-bentazone (labeled in position 10) was investigated in four soils at room temperature. The application rates were 2 kg/ha for all soils. Additionally, the influences of application rate, soil temperature, moisture and pH were determined for 4 soils, with the unlabbeled a.s.

Soil characteristics:

soiltype	Clay [%]	Silt [%]	Sand [%]	org.C [%]	pH	MWHC [%]	CEC [meq/100g]
Loamy sand	5.5	14	80.5	0.8	6.7	28	5.2
Clay loam	24	22	54	3.1	7.5	40	25.6
Clay loam	12	37	51	0.9	5.1	41	10
Loamy sand	6	8	86	2.7	4.6	41	4.0

Results

After 100 days about 80 % of as was degraded, followed by very slow further degradation. After one year 80 % of non-extractable residues were formed. Carbon dioxide was formed up to 2 % after 60 days. The extractable radioactivity represents mainly the as, but after 60 days the metabolite 2-amino-isopropyl-benzamide (AIBA) which was identified by thin layer chromatography occurred (0.02 ppm). The degradation rate of AIBA is significantly faster than that of as.

Influence of the different parameters:

Application rate: The degradation decreased in relation to the application rates: 2 kg/ha as degraded in 10 weeks, 5 kg/ha as in 15 weeks and 10 kg/ha in 25 weeks.

Soil temperature: The effect of three temperatures 10, 23 and 36°C on the degradation of bentazone in soil was investigated. The initial concentration of bentazone in the soil was 2mg/kg. The soil moisture was maintained at 42% field capacity and the soil was kept at 10, 23 and 36°C. Bentazone residues were extracted with methanol and determined by GLC. The differences between 23 and 3 6°C are minor. However, the degradation of bentazone (DT50) at 10 °C was decreased from about 35 days to 161 days. This result was derived by graphical interpolation.

Soil moisture: Degradation rates were not influenced by this parameter (maximum water holding capacities: 18-54%).

pH: The degradation rate decreased meaningful at pH 4 and 6.

Comment

Acceptable.

RMS comment 2013: The studies were considered acceptable in the original DAR however, based on the reduced information vailable in the original report and the short summary presented in the original DAR RMS cannot confirm this study can still be considered acceptable, even when the requirements with regard to study quality at the time of the original DAR are taken in consideration.

reference	:	Drescher and Otto, BOD96-00055, BOD96-00057	GLP statement	:	no
Type of study	:	Soil degradation	Guideline	:	unknown
Year of execution	:	1973	Acceptability	:	Not acceptable
test substance	:	^{14}C-bentazone	Previous evaluation	:	In original DAR: acceptable

Method:

The experiments were conducted to further study the metabolism of ^{14}C-bentazone (labeled in position 10) (see Method above).

Soil characteristics:

soiltype	Clay [%]	Silt [%]	Sand [%]	org.C [%]	pH	MWHC [%]	CEC [meq/100g]
Loamy sand	6	8	86	2.7	4.6	41	4.0

Results

Bentazone degradation is clearly aerobic. In a nitrogen atmosphere degradation is completely stopped, but starts again if the soil is exposed to air. AIBA (see above) is formed in the soil only to a limited degree (about 0.01 ppm), and it does not accumulate. At the end of the study (22 weeks) almost the whole ^{14}C-activity added to the soil in form of ^{14}C-bentazone was present as a soil bound, methanol insoluble residue.

Comment

Acceptable.

RMS comment 2013: The studies were considered acceptable in the original DAR however, based on the reduced information vailable in the original report and the short summary presented in the original DAR RMS cannot confirm this study can still be considered acceptable, even when the requirements with regard to study quality at the time of the original DAR are taken in consideration.

reference	:	Drescher and Otto, BOD96-00056	GLP statement	:	no
Type of study	:	Soil degradation	Guideline	:	unknown
Year of execution	:	1973	Acceptability	:	Not acceptable
test substance	:	^{14}C-bentazone	Previous evaluation	:	In original DAR : acceptable

Method:

The experiments were conducted to further study the metabolism of bentazone (labeled in position 10) (see Methods studies above).

Soil characteristics:

soiltype	Clay [%]	Silt [%]	Sand [%]	org.C [%]	pH	MWHC [%]	CEC [meq/100g]
Clay loam	24	22	54	3.1	7.5	40	25.6

Results:

At the end of the investigation (38 weeks) non-extractable residues were formed up to 70 %. A part of these residues were not extractable with alkaline solutions. It is assumed that these residues are located in the humins.

Comment:

Acceptable.

RMS comment 2013: The studies were considered acceptable in the original DAR however, based on the reduced information vailable in the original report and the short summary presented in the original DAR RMS cannot confirm this study can still be considered acceptable, even when the requirements with regard to study quality at the time of the original DAR are taken in consideration.

reference	:	Keller, BOD95-00264	GLP statement	:	no
Type of study	:	Soil degradation	Guideline	:	unknown
Year of execution	:	1987	Acceptability	:	Not acceptable

test substance	:	^{14}C-bentazone (phenyl-u-14C)	Previous evaluation	:	In original DAR : acceptable

Method:

The aerobic metabolism pathway for ^{14}C-bentazone (phenyl-u-14C) was investigated in three soils. For the fortification an initial concentration of 10 mg as/kg soil was used, which is equivalent to about 10 kg/ha. This "high" initial concentration was chosen so that even residues amounting to 1 % of the initial radioactive residue could be identified. After spiking and thoroughly mixing the soils were adjusted to 40% maximum water holding capacity. The soil reactor (all glass) consisted of a metal rack with petri dishes filled with soil. It was purged with a constant $C0_2$ free moist air flow of ca. 0.5 1/h. Each of the soil trays was filled with approximately 100 g soil. Each tray soil reactor filled with 10 trays at maximum. In order to measure the mineralization rate the absorption solutions in the three traps (ethylene glycol, H_2S0_4, PPBAX cocktail) were changed periodically and aliguots counted by LSC. Samples were taken 0, 3, 7, 14, 28, 60, 90, 180, 270 and 360 days after beginning of the incubation. The moisture levels were determined by weighing at sampling and readjusted by addition of water, if necessary. The soil samples were analyzed either immediately after sampling or kept deep frozen at - 20 °C until analysis. The soils were extracted three times by shaking the samples with methanol (or some with acetone) at room temperature for some ten minutes. Some additional extraction were done by extracting the relevant samples with acetone instead of methanol. When the residues of the soil surpassed ca. 10 % of the initial radioactive residues these fractions were further worked up (extraction: three times with 0.1 n HCl in methanol, three times with water (occasionally), further extraction with 0.5 N NaOH (three times). In each case, phase separation was done by filtration and/or centrifugation. The extracts were evaporated at ca. 50°C and redissolved in small amounts of methanol for radio-TLC analyis, or sometimes redissolved in water and extracted with ethylacetate at pH 1-2.

Soil characteristics:

soiltype	Clay [%]	Silt [%]	Sand [%]	org.C [%]	pH	MWHC [%]	CEC [meq/100g]	Microb.biomass [mg C/100g]	
								start	end
Sandy loam	11.9	16.1	72	0.5	6.1	52	10.6	19.6	10.7
Loamy sand	7.8	6.9	85.3	0.6	5.0	31	3.4	11.2	<5
Clay	24.2	29.4	46.4	2.9	7.7	61	29.6	35.6	52.4

Analytical procedures: Liquid samples were radioassayed by liquid scintillation counting, solid samples were combusted and the evolved $^{14}C0_2$ absorbed in a suitable scintillation cocktail for radioassay. Radio-TLC was used for the detection of degradation products of bentazone. "Cold" standards were cochromatographed and visualized with UV-light. Preparative TLC was done to prepurify samples. Other chromatographic systems involved radio-HPLC, column chromatography, gas chromatography and MS (direct inlet) or GC/MS. Prior to amenating samples to GC they were derivatized e.g. by diazomethane or employing phase transfer catalysis.

Test conditions:

Soil no.	conditions	Temperatures ºC	Application rate mg/kg
5-7	Aerobic	22 ± 2	9.46/9.48/10.94

Results :

^{14}C-Recoveries (in %)

Soil	5	6	7*)
Min	83.0	89.7	77.2
max	98.9	110.7	87.8

*) The poorer balances are explained by sample inhomogenities.

At the first samplings, only as could be detected (i.e. in sandy loam sample, unter 90 days, in loamy sand saple, until 60 days, and in clay soil sample, also until 60 days). During laboratory tests non-extractable residues amounted to 43 - 74 % of dose applied after 90 days (see diagram B.7.1-1, table B.7.1-2). So the main pathway for the aerobic degradation of bentazone in three soils was the incorporation into humic substances, and even into humus fractions which were not extractable by aqueous NaOH. Most probably, not bentazone itself, but degradation intermediates (e.g. hydroxybentazones or other reactive intermediary products) were bound to these fractions.

Minor pathways were the methylation and halogenation (prevailing chlorination, but also bromination) of the active substance. Both reactions are known to be performed by soil microorganisms, e.g. antibiotics like chloroamphencol, grisefulvin and pyrrolnitrin (containing one or more chlorine atoms) are formed by Stryptomyces venezuelae, Penicillium griseofulvum and Pseudomonas pyrrocinia, respectively. Besides bentazone, 8-chlorobentazone (< 1.6 %) and N-methylbentazone (< 2.6%) components in ultra trace amounts such as chlorobentazone and bromobentazone, probably substituted in the 6-position, were found by GC/MS analysis. The substance 2-amino-isopropyl-benzamide (AIBA) which is a formerly postulated soil metabolite of bentazone was not found in this study with active soils.

The mineralization rates were 6 - 9 % after 90 days and increased up to 12 - 24 % at the end of the studies.

Comment:

Acceptable.

RMS comment 2013: The studies were considered acceptable in the original DAR however, based on the reduced information vailable in the original report and the short summary presented in the original DAR RMS cannot confirm this study can still be considered acceptable, even when the requirements with regard to study quality at the time of the original DAR are taken in consideration.

One additional study is available which was already peer reviewed in the EU process and included in the "Addendum to the Monograph – Volume 3, 17 May 2000": Ebert (2000). This study is copied below.

reference	:	Ebert, D., BOD 2000-496	GLP statement	:	yes
Type of study	:	Degradation of Bentazon (BAS 351 H) in Lysimeter Soil (Borstel, Northern Germany),	Guideline	:	SETAC (1995)
Year of execution	:	2000	Acceptability	:	acceptable
test substance	:	^{14}C-Phenyl]-bentazone	Previous evaluation	:	In original DAR; addendum

Test system

The degradation of [^{14}C-Phenyl]-bentazone (chemical purity 99.6 %, radiochemical purity >99 %) was investigated in the soil Boden Borstel, using the soil layer 0-34 cm (Loamy Sand, 78 % sand, 17 % silt, 5 % clay, 1.1 % Corg, CEC 10 meq/100 g, pH-$CaCl_2$ 5.6, field capacity at 330 hPa: 9.3 %). 1.3 mg as/kg was applied (=1 kg as/ha) and incubated. Soil samples were analysed on day 0, 3, 7, 14, 30, 60, 91 and 183 after treatment. The volatiles at 20 °C and 40 % MWHC were trapped in a trapping system of three gas washing flasks containing (1) 0.5 m NaOH, (2) 0.5 M $H_2SO_4$and (3) ethylene glycole. The soil was extracted three times with methanol followed by 3 extraction steps using methanol : water (1:1). The radioactivity of soils samples were determined after extraction by combustion and the radioactivity in the extracts were measured by LSC. HPLC was used to determine the amounts of bentazone and metabolites. The following substances were used as reference substances (co-chromatography): N-methyl-bentazone, 6-OH-bentazone and 8-OH-bentazone. First order kinetics was fitted to the data using Modelmaker.

Results:

Table B.7.1-1 shows the distribution of the radioactivity in the incubation system. The mass balances range between 96.5 and 105.6 % TAR. After 91 days 10.6 % of the applied radioactivity was detected as CO_2 and 73.4 % as bound residues. At the end of the study (day 183) 11.7 % of the radioactivity were determined as CO_2 and 75.0 % TAR as bound residues. The amounts of the substances identified are listed in Table B.7.1-2. One metabolite (N-methyl bentazone) could be identified with a maximum amount of 5.8 % TAR on day 183 (5.5 % TAR on day 91). All other peaks in sum not exceeded 4 % TAR at any sampling point. The degradation of bentazone can be described well by 1st order kinetics. A DT50 value of 16.9 days and a DT90 value of 56.2 days were estimated (r^2 = 0.99).

Table B.8.1.1-01: Aerobic degradation of bentazone – distribution of the radioactivity in the system (% of the applied radioactivity)

Day	Extractables (%)	Bound residues (%)	CO_2 (%)	Total (%)
0	102.6	2.9		105.5
3	83.3	16.7	0.3	100.2
7	74.8	30.0	0.9	105.6
14	56.5	39.0	1.9	97.4
30	34.0	59.9	4.5	98.4
60	16.4	73.6	8.0	98.1
91	12.5	73.4	10.6	96.5
183	11.2	75.0	11.7	97.9

Table B.8.1.1-02: Aerobic degradation of bentazone – amounts of bentazone and metabolites detected (% of the applied radioactivity)

Day	Bentazone (% TAR)	N-methyl Bentazone (% TAR)	Others (% TAR)
0	99.6	0.5	
3	79.1	0.5	
7	69.2	0.8	
14	54.5	1.9	

30	31.0	3.0	
60	5.9	5.5	3.7
91	1.2	5.5	3.1
183	0.3	5.7	3.4

Comment:

The study is acceptable. The amount of bound residues exceeded 70 %, but the mineralisation rate is higher than 5 % within 100 day (~11 %).

A kinetic evaluation of the study according to FOCUS (2006) was provided upon request during the peer review process.

STUDY IIA, 7.1.1/00

Characteristics

reference	:	Budde, E., 2014(a)	Study type	:	IKinetic evaluation
year of execution	:	2014		:	
GLP statement	:	Not relevant, GMP		:	
guideline	:	FOCUS Kinetics (2006)	DT50	:	See results
test substance	:	BAS351H	DT90	:	See results
Six field trials	:	Borstel	acceptability	:	**acceptable**

Study design

The purpose of this evaluation was to analyze the degradation kinetics of bentazone and its metabolite N-methyl-bentazone observed in the soil, taking into account the current guidance of the FOCUS workgroup on in order to derive degradation parameters as triggers for additional work (trigger endpoints) and for modeling purposes (modeling endpoints). For completeness the soil parameters of the test soil are given as the original summary of the report in the addendum was not complete to this regard.

Soil designation	Borstel
Origin	Borstel, Germany (0-34 cm)
Soil type (USDA)	Loamy sand
Particle size distribution [%] sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm	 79.6 15.1 5.3
Total organic C [%]	1.1
pH ($CaCl_2$)	5.6
Cation exchange capacity [meq Ba/100 g]	10
Soil moisture at pF 2.5 (0.33 bar) [g/100g dry soil]	9.3
Maximum water holding capacity [g H_2O/100 g soil]	29
Microbial biomass [mg C/100g dry soil]	20.7

The experimental data were derived from the study report (Ebert, 2000) and adjusted according FOCUS . The metabolite N-methyl-bentazone occurred in amounts >5% during the study and was therefore included in the evaluation. The software package KinGUI (version 2.2) was used for parameter fitting. The DegT50 values suitable for modeling were normalized to reference conditions (temperature of 20°C and soil moisture at field capacity, i.e. pF2). Since the study was performed at 20°C a temperature correction was not necessary. The moisture normalization was performed using the moisture dependency equations by Walker.

Results

The visual fits for the parent only fits are presented in figure A1 and A2. The parameter results are presented in table 1.

Figure A 1 Kingui fit for parent bentazone in soil Borstel, SFO kinetics

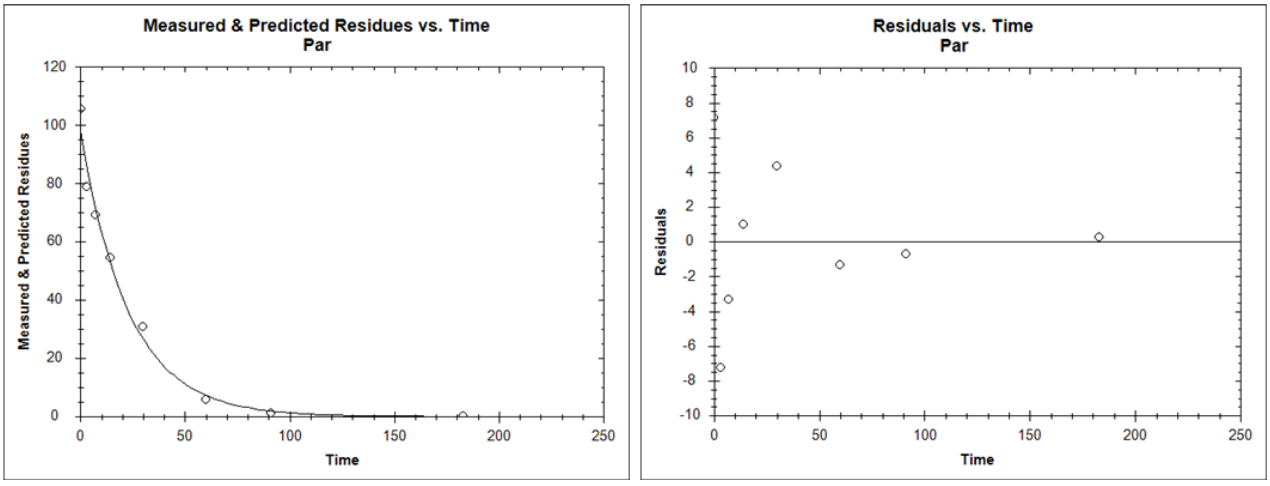
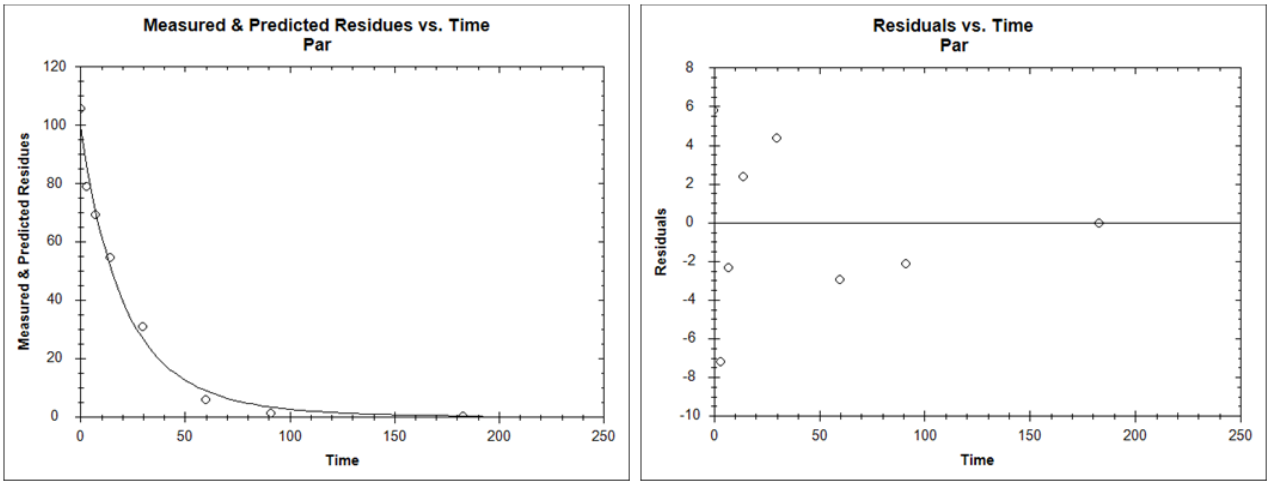


Figure A 2 Kingui fit for parent bentazone in soil Borstel, FOMC kinetics



Following the metabolite was included in the fitting procedure using SFO-SFO kinetics. The visual fits are presented in figure A3.

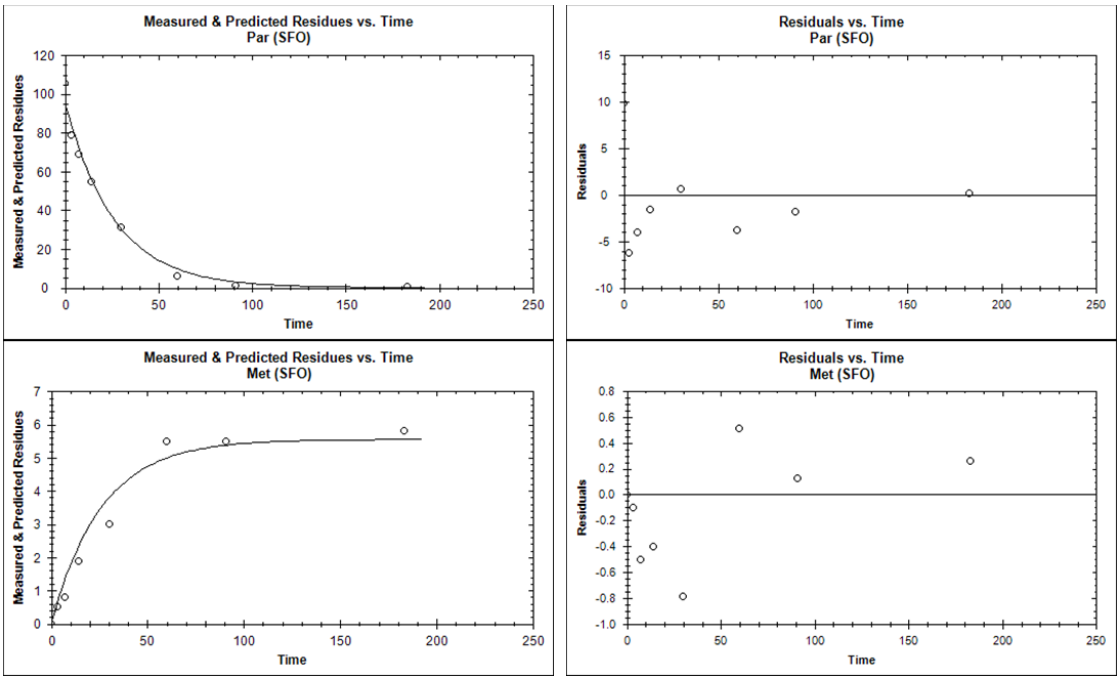


Table 1Statistical and visual assessment of different kinetic models for bentazone and N-methyl-bentazone in Borstel soil

Kinetic model	**χ^2 error**	**Estimated parameter**	**p (t-test)**	**Visual assessment**	**DT_{50} [d]**	**DT_{90} [d]**
SFO	7.6	M0: 98.35 k: 0.0435	k: <0.001	Good	15.9	52.9

FOMC	7.9	M0: 99.68 α:6.63 β:136.3	α: not sign β: not sign	Good	15.0	56.6
- The SFO visual fit is good, residuals are randomly scattered around zero, the χ^2 error value is low, k is significantly different from zero. - The FOMC model did not improve the visual fit, the χ^2 error value is slightly higher, the parameter β is not significantly different from zero. - **Conclusion**: Use **SFO** to derive **modeling** and **trigger endpoints** for the parent.						
Parent SFO	8.5	M0: 95.7 k: 0.038	k: <0.001	Good	18.1	60.2
Metabolite SFO	10.8	k:1.7x10^{-8}	k: <0.001	good	Not significant	Not significant
- The SFO visual fit for metabolite is good, the value of χ^2 error is low, but the k-rate is not significantly estimated. - The metabolite formation is adequately described by the SFO model (plateau is reached). - **Conclusion**: The parent-metabolite fit is reliable. Use **parent-metabolite fit** (SFO-SFO) to derive **modeling** and **trigger endpoints** for the parent. For the metabolite, a reliable formation fraction could be estimated (ff = 0.058).						

The values derived for parent substance were normalised to reference conditions to derive endpoints suitable for modelling. Temperature correction was not necessary since the study was performed at

20°C. Parameters included in the normalization procedure and the resulting DegT50 value for

modeling are summarized in Table 2.

Table 2Normalisation of the bentazone DegT50 value to reference conditions

soil	**Kinetic model**	**θact**	**θref**	**fmoist**	**DegT50act [d]**	**DegT50ref [d]**
Borstel	**SFO**	**11.6**	**14**	**0.877**	**18.1**	**15.9**

θact actual soil moisture (40% of MWHC) [g / 100 g dry soil]

θref reference soil moisture at field capacity (pF 2) [g / 100 g dry soil]

fmoist moisture correction factor [-]

DegT50act DegT50 at study conditions [d]

DegT50ref DegT50 at reference conditions [d]

Conclusion

A summary of the trigger and modeling endpoints for bentazone calculated from the Borstel soil is given in Table 3.

Table 3 Summary of trigger and modeling endpoints for bentazone

Soil	**Kinetic model**	χ^2	**Trigger endpoints**		**Modeling endpoint**
			DT_{50}	**DT_{90}**	**Normalized DegT50 [d]**
Borstel	SFO	8.5	18.1	60.2	15.9

The metabolite N-methyl-bentazone was observed with a maximum of about 6% TAR at the end of the study. The metabolite formation could be adequately described by the SFO model, with a formation fraction of 0.058, but no reliable degradation rate for the metabolite could be derived.

STUDY IIA, 7.1.1/01

Characteristics

Reference	:	Staudenmaier H., Kuhnke G. 2010b	study type	:	aerobic degradation
year of execution	:	2009-2010	incubation time	:	up to 150 d
GLP statement	:	yes	nominal concentration	:	2.7 mg/kg
Guideline	:	Incl. OECD 307 (2002)	temperature	:	20°C
test substance	:	Bentazone Lablled: phenyl-U-^{14}C, Batch 2010-2301	DT50	:	see results

Purity	:	radiochemical purity 96.5%.	metabolites	:	see results
Soil	:	sandy loam	acceptability	:	**acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Study design

The aerobic soil metabolism of bentazone was investigated in a sandy loam (Bruch West, Limburgerhof, Germany) freshly collected from the field. Soil characteristics are reported in table B.8.1.1-03 below. The soil was treated with [^{14}C-phenyl]-bentazone at a nominal rate of 2.7 mg per kg dry soil which corresponds to a field application rate of 1000 g bentazone per hectare, calculated on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g/cm³.

Table B.8.1.1-03 Properties of soil Bruch West used to investigate degradation of [phenyl-U-^{14}C]- bentazone under aerobic conditions

Soil designation	**Bruch West (09/060/03)**
Origin	Limburgerhof, Germany
USDA Particle size distribution [%] sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 63.8 24.9 11.2 sandy loam
Organic C [%] Organic matter [%] *	1.23 2.12
pH (H_2O) pH ($CaCl_2$)	7.9 7.3
Cation exchange capacity [$cmol^+$ / kg]	12.2
Maximum water holding capacity [g/100g dry soil]	23.6
Microbial biomass (before start of study) [mg C/100g dry soil]	37.0

Microbial biomass (after 57 days) [mg C/100g dry soil]	31.7**
Microbial biomass (after 126 days) [mg C/100g dry soil]	28.4**

* organic matter = organic carbon x 1.724

** determined at BASF test facility Limburgerhof

Soil aliquots of 100 g (dry weight basis) were weighed into test vessels and incubated in the dark under aerobic conditions at soil moisture of 40% of the maximum water holding capacity and a temperature of 20°C. A closed incubation system with continuous aeration (moistened air) was used with an attached trapping system for the collection of volatile compounds. At two time points during incubation (57 and at 126 DAT), the microbial biomass was determined by the substrate induced respiration method, verifying that the soil was viable throughout the incubation period.

Samples were taken at 0, 1, 3, 7, 14, 30, 64, 91, 120 and 150 days after treatment (DAT).

The soil samples were extracted twice with methanol and twice with water/methanol (v:v, 1:1). The amount of radioactivity in the individual extracts was determined by liquid scintillation counting (LSC). The methanol and water/methanol extracts per soil sample were combined, respectively, and analysed by means of HPLC. The remaining soil was homogenized and combusted after extraction to determine the amount of non-extractable residues (NER) in soil. The NER were further characterized by NaOH extraction and subsequent fractionation into fulvic acids, humic acids and humins. The fulvic acid fraction was furthermore partitioned with ethyl acetate. A full material balance was provided for each sampling interval.

Results

The results showed that the amount of extractable radioactivity in soil continuously decreased from 102.2% of the total applied radioactivity (TAR) at 0 DAT to 6.9% TAR after 150 days of incubation. The amount of the test item bentazone decreased from 101.0% TAR at 0 DAT to 2.3% TAR at 150 DAT. Metabolites were formed only in minor amounts of which the most prominent metabolite (max. 2.8% TAR) was identified as N-methyl-bentazone (M351H009). All other metabolites were formed in even lower amounts and their sum in the total extracts never exceeded 2.2% TAR at any sampling time.

Mineralisation to ^{14}C-CO_2 reached a total of 9.0% TAR after 150 days of incubation. No other volatile compounds were detected.

The amount of non-extractable radioactive residues (NER) increased during the course of the study from 3.3% TAR on day 0 to 68.8% TAR at the end of the study after 150 days. After extraction with NaOH, still about half of the radioactivity (2.3 – 34.8% TAR) remained tightly bound to the soil matrix (humines). The NaOH extractable radioactivity was distributed between the humic acid (1.0 – 11.9% TAR) and the fulvic

acid fraction (2.7 – 21.0% TAR) in a ratio of about 1:2. The fulvic acid fraction was further characterized by partitioning with ethyl acetate. Amounts of 1.6 to 7.3% TAR of the fulvic acid fraction were soluble in ethyl acetate, whereas 0.9 to 13.1%TAR remained in the water phase. The ethyl acetate soluble fractions from samples of 30 DAT to 120 DAT were investigated by HPLC. Parent compound was found to be the most prominent peak, accounting for 3.0 to 3.7% TAR. Mineralisation of ^{14}C-bentazone in soil was moderate mineralization, with CO_2 amounts reaching a total of 9.0% TAR after 150 days of incubation. No other volatile compounds were observed.

The material balance throughout the incubation period ranged from 92.6 to 105.5% TAR except for the 150 DAT sampling, for which a material balance of only 84.7 TAR was achieved. The average material balance for all soil samples was 98.0% TAR.

The results are summarised in table B.8.1.1-04 and B.8.1.1-05 below.

Table B.8.1.1-04 Distribution of radioactivity in soil Bruch West after treatment with [phenyl-U-^{14}C]-bentazone and aerobic incubation at 20°C [%TAR]

Days after treatment	Extractable			NER	Volatiles		Material balance
	Methanol	Methanol / H_2O	Total		CO_2	Other volatiles*	
0	86.0	15.0	101.0	3.4	n.d.	n.d.	104.4
0	88.4	15.0	103.4	3.3	n.d.	n.d.	106.6
0 mean	87.2	15.0	102.2	3.3	n.d.	n.d.	105.5
1	84.8	13.4	98.2	5.8	0.1	0.0	104.1
3	85.9	11.0	96.9	8.8	0.3	0.0	106.0
7	81.2	10.6	91.8	11.4	0.7	0.0	103.9
14	73.6	10.1	83.8	15.3	1.2	0.0	100.3
30	60.7	11.0	71.6	24.6	2.3	0.0	98.6
30	61.5	10.7	72.2	25.1	2.3	0.0	99.6
30 mean	61.1	10.8	71.9	24.9	2.3	0.0	99.1
64	37.6	8.6	46.1	49.0	4.4	0.0	99.5
91	24.4	6.1	30.5	56.9	6.5	0.0	93.9

Days after treatment	Extractable			NER	Volatiles		Material balance
	Methanol	Methanol / H_2O	Total		CO_2	Other volatiles*	
120	15.4	4.7	20.1	64.7	8.2	0.0	93.0
120	15.3	4.7	20.0	63.9	8.2	0.0	92.2
120 mean	15.3	4.7	20.1	64.3	8.2	0.0	92.6
150	4.3	2.2	6.5	66.9	9.0	0.0	82.4
150	4.9	2.3	7.2	70.7	9.0	0.0	86.9
150 mean	4.6	2.3	6.9	68.8	9.0	0.0	84.7

* = sum of radioactivity in H_2SO_4 and ethylene glycol traps

n.d. = not determined

NER = non-extractable residues

TAR = total applied radioactivity (100% = 2.6839 mg/kg dry soil)

Table B.8.1.1-05 Radio-HPLC-analysis of soil extracts after treatment of soil Bruch West with [phenyl-U-^{14}C]-bentazone and aerobic incubation at 20°C [%TAR]

days after treatment	^{14}C total extractable	Bentazone (BAS 351 H) t_R ~35.8'	N-methyl-bentazone (M351H009) t_R~44.9'	Sum others
0	101.0	99.7	n.d.	1.2
0	103.4	102.2	n.d.	1.2
0 mean	102.2	101.0	n.d.	1.2
1	98.2	97.2	n.d.	1.0
3	96.9	95.8	n.d.	1.1
7	91.8	91.0	n.d.	0.8

14	83.8	83.0	n.d.	0.8
30	71.6	70.1	0.6	0.9
30	72.2	70.6	0.6	1.0
30 mean	71.9	70.4	0.6	0.9
64	46.1	43.2	1.7	1.3
91	30.5	26.6	2.2	1.7
120	20.1	15.4	2.7	2.0
120	20.0	15.0	2.9	2.1
120 mean	20.1	15.2	2.8	2.0
150	6.5	2.1	2.4	2.0
150	7.2	2.4	2.3	2.4
150 mean	6.9	2.3	2.4	2.2

t_R = retention time [min]

Table B.8.1.1-06 Characterization of non-extractable residues in soil Bruch West after treatment with [phenyl-U-^{14}C]-bentazone and aerobic incubation at 20°C [%TAR]

days after treatment	**NER**	**NaOH extract**	**Fulvic acids**			**Humic acids**	**Humins**
			total	**Ethyl acetate soluble**	**Acidic water soluble**		
1	5.8	3.6	2.7	1.6	0.9	1.0	2.3
3	8.8	5.0	3.4	1.9	1.4	1.5	3.8
7	11.4	6.4	4.3	2.3	1.8	1.9	5.1
14	15.3	8.3	5.7	3.0	2.5	2.5	7.1
30*	24.6	12.9	8.5	4.2	4.1	4.0	12.7
64	49.0	26.0	16.6	6.8	9.1	8.8	23.6
91	56.9	31.2	19.3	6.6	12.1	10.8	30.4
120*	64.7	34.1	21.0	7.3	13.1	11.9	34.8

* replicate 1

n.d. = not determined

NER = non-extractable residues

TAR = total applied radioactivity (100% = 2.6839 mg/kg dry soil)

Kinetic analysis and calculation of DT_{50} and DT_{90} values for bentazone was performed following the recommendations of the FOCUS Kinetics workgroup. The analysis was done by non-linear regression methods using the software package KinGUI version 1.1.

The estimated DT_{50} and DT_{90} values of bentazone in aerobic soil are shown in Table B.8.1.1-07.

Table B.8.1.1-07 Estimated DT_{50} and DT_{90} values and χ^2 - error level

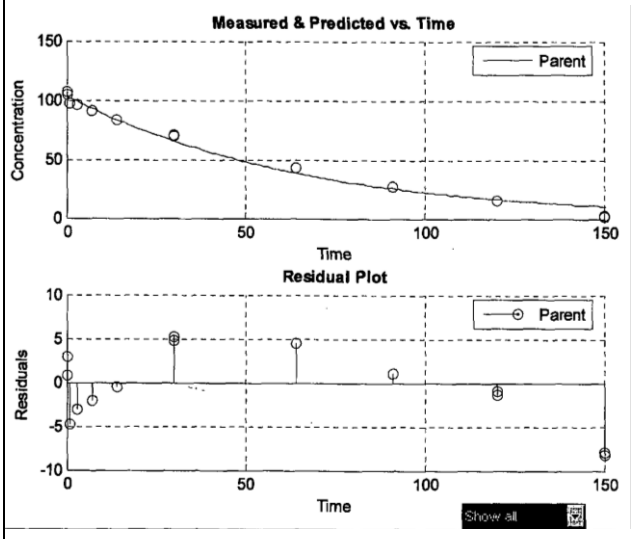
Kinetic model	Substance	DT_{50} [d]	DT_{90} [d]	χ^2 - error level [%]	Visual fit
SFO*	Bentazone	45.1	149.8	5.4*	Good
FOMC	Bentazone	44.8	150.6	5.7	Good

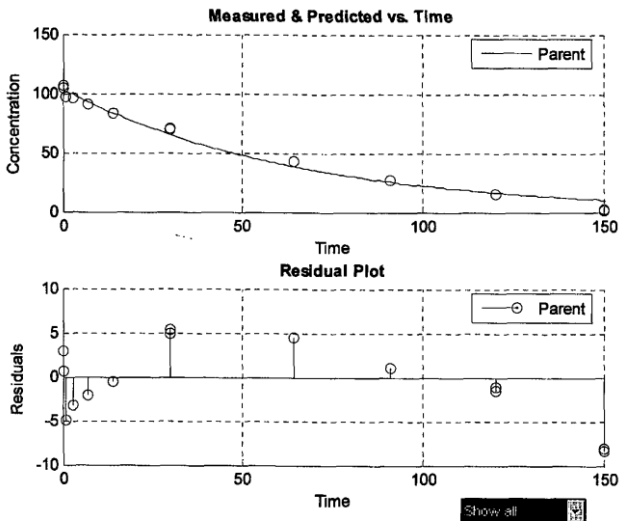
*: best fit model

The corresponding plots of modelled against observed residues are presented in Figure B.8.1.1-01.

Figure B.8.1.1-01 ^{14}C-phenyl-labeled bentazone: modelled against observed residue

Bentazone - SFO **Bentazone - FOMC**





The FOMC model was not more appropriate than the SFO model The SFO model provided an excellent visual fit and a low χ^2 error.

Conclusion

From the results of the present study, it is concluded that bentazone degrades relatively fast in soil Bruch West when incubated under aerobic conditions at a soil moisture of 40% of the maximum water holding capacity and a temperature of 20°C. Metabolites were formed only in very low amounts. Even the most prominent metabolite N-methyl-bentazone (M351H009) never exceeded 2.8% TAR.

Characterization of the non-extractable radioactive residues (NER) revealed that about half of the radioactivity was tightly bound to humins, while the rest was distributed in a ratio of about 1:2 between the humic acid and the fulvic acid fractions. Only by harsh extraction methods leading to destruction of the humic structure and subsequent analysis of the fulvic acids, a maximum of 3-4% of the overall 65% TAR bound residues could still assigned to bentazone. $^{14}CO_2$ was formed in low amounts reaching 9% TAR at the end of the study after 150 DAT. No other volatile compounds were detected.

The SFO DT_{50} and DT_{90} values were 45.1 days and 149.8 days, respectively. These values are appropriate for use as persistence endpoints as well as modelling endpoints after normalisation to reference moisture conditions.

Comment

The study is acceptable and the results can be used for risk assessment.

Second notifier data

Study was part of the equivalent dossier and was submitted with the renewal dossier.

STUDY IIA, 7.1.1/02

Characteristics

reference	:	Völkel W. 2001	study type	:	aerobic degradation
year of execution	:	2001	incubation time	:	up to 117 d
GLP statement	:	yes	nominal concentration	:	1.46 mg/kg
guideline	:	I OECD Draft Aerobic and Anaerobic Transformation in Soil. (2000)	temperature	:	20 ±2 °C
test substance	:	Bentazone Label position unclear: ring-U-^{14}C, Batch CFQ11197 (RCC No. 95841)	DT50	:	see results

purity	:	Unlabbel substance chemical purity 99.9%; radiochemical purity 99.8%.	metabolites	:	see results
soil	:	loamy sand	acceptability	:	**acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Study design

Degradation of ^{14}C-bentazone, i.e. 3-isopropyI-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2 dioxide, was studied in Borstel soil (see table for soil characteristics) under aerobic conditions, for a period of up to 117 days. Soil samples (100 g dry weight) were treated corresponding to 1.46 mg/kg soil dry weight, which corresponds to the maximum recommended field rate of 1.5 kg a.i./ha, calculated on the basis of an equal distribution in the top 1 cm soil layer and a soil density of 1 kg/m³. Soil samples were incubated at 20 °C ± 2 °C and at about 40% of maximum water holding capacity. The flasks were equiped with an air inlet and outlet. The system was continuously ventilated with moistened air and outgoing air was passed through a trapping system, equiped with absorption traps containing 50 ml of ethylene glycol and of 50 ml 2N NaOH, in this order, to trap organic volatiles and ^{14}CO2, respectively. Samples were taken for analysis after 0, 1, 3, 7, 14, 30, 56 and 117 days.

Table B.8.1.1-08 soil characteristics

Parameter *		Borstel 98
Batch		Same as RCC 719728
pH	(KCl)	5.6
Organic carbon	g/100 g dry soil	1.77
Carbonate	% $CaCO_3$	1.4
Cation exchange capacity	meq/100 g dry soil	7.12
Particle size (mm)		
Classification		Loamy sand **
<0.002	clay	6.47
0.002 - 0.05	silt	12.65
>0.05	sand	80.88
Max. water holding capacity	g/100 g dry soil	30.94
40% MWHC	g/100 g dry soil	12.4
Biomass ***	mg C/100 g dry soil	
a) Start of incubation		27.7
b) Completion of incubation		24.1

* Parameters: as determined by AgroLab , CH-6031 Ebikon (non-GLP).

** Soil type according to USDA

*** Biomass determined within this study

Samples were extracted with:

a) Acetonitrile/water (99.5:0.5, v/v), 2-3 times

b) Soxhlet with acetonitrile/water (9:1, v/v), for at least four hours (day 0 to day 30 samples)

c) Reflux with acetonitrile/0.1 M HCI (1:1, v/v) for about two hours (beginning with 30 day sample).

Extracts were concentrated and analysed by HPLC and selected extracts were further analysed

by TLC. The recovered radioactivity after concentration of the extracts was determined by LSC and

ranged between 92.7% and 101.6%. The residual radioactivity remaining in soil after all extraction procedures was quantified by LSC after combustion

The rate of disappearance of ^{14}C-bentazone in soil was calculated by applying a one compartment first-order model.

Results

The total recoveries averaged 95.1% ± 2.3% of the applied radioactivity. The extracted radioactivity at room temperature decreased from 92.8 % (day 0) to 36.5% of the applied radioactivity on day 14 and represented 9.0% of the applied radioactivity on day 117. The amount of non-extractable radioactivity increased from 0.8% AR on day 0 to 60.8% on day 56 and 53.5% at the end of incubation (day 117).

Mineralization of ^{14}C-bentazone and its residues to $^{14}CO_2$ amounted to 14.9% of the applied radioactivity at the end of incubation. Other volatiles did not exceed 0.1% of the applied radioactivity. Results of extractions are summarised in table B.8.1.1-09.

Table B.8.1.1-09 Material balance in soil. Values given in %AR

Bentazone (Balance) (% applied)	INCUBATION TIME IN DAYS							
	0	1	3	7	14	30	56	117
Extractables*	92.8	87.3	77.0	59.0	36.5	14.5	7.4	9.0
Soxhlet**	2.8	3.8	3.5	3.0	5.3	2.8	n.p.	n.p.
Reflux***	n.p.	n.p.	n.p.	n.p.	n.p.	13.4	18.8	14.6
Total Extractables	**95.6**	**91.1**	**80.6**	**62.0**	**41.7**	**30.6**	**26.2**	**23.6**
Non-Extractables	0.8	6.5	14.9	31.1	49.4	53.8	60.8	53.5
$^{14}CO_2$	n.p.	n.p.	0.4	1.4	3.5	7.6	10.9	14.9
Other Volatiles in ethylene glycol	n.p.	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
TOTAL	**96.5**	**97.6**	**95.8**	**94.6**	**94.6**	**92.0**	**98.0**	**92.0**
Mean ± SD				**95.1**	±	**2.3**		

*: Extracted with acetonitrile/water 99.5:0.5 (v/v)

**: Soxhlet with acetonitrile/water 9:1 (v/v)

*** Reflux with acetonitrilel/0.1 M HC11:1 (v/v)

n.p.: Not performed

Table B.8.1.1-10 ^{14}C-bentazon and degradation products in soil extracs. Values given in percent of applied radioactivity

(Balance) Parent (Bentazone) (% applied)	INCUBATION TIME IN DAYS							
	0	1	3	7	14	30	56	117
Parent (Bentazone)	95.6	91.1	79.8	60.3	37.8	10.9	2.4	1.3
M1	*	*	*	*	0.3	0.6	0.6	0.8
M2	*	*	*	0.2	0.4	0.8	0.4	1.1
M3	*	*	0.8	0.9	1.9	1.8	1.8	0.8
M4	*	*	*	*	0.3	0.7	0.1	0.8
M5	*	*	*	*	1.0	0.3	0.2	0.2
M6	*	*	*	0.7	*	2.1	1.9	2.8
M7**	*	*	*	*	*	13.4	18.8	14.6
M8	*	*	*	*	*	*	*	1.1
M9	*	*	*	*	*	*	*	0.1
Non-Extractables	0.8	6.5	14.9	31.1	49.4	53.8	60.8	53.5
$^{14}CO_2$	n.p.	n.p.	0.4	1.4	3.5	7.6	10.9	14.9
TOTAL	96.5	97.6	95.8	94.6	94.6	92.0	98.0	92.0

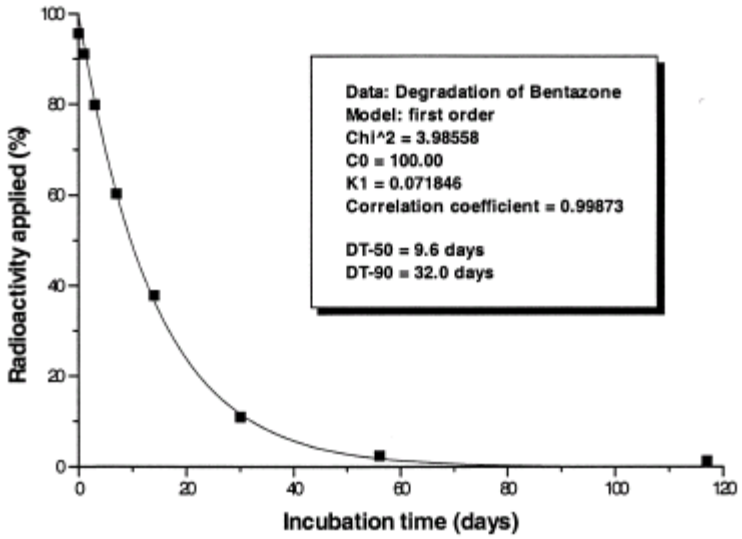
* Not detected or below the limit of quantification.

** M7: radioactivity extracted by harsh extractions. This fraction consists of at least 4 radioactive fractions M7-1,*2,3,4* as indicated by TLC analysis (Figure 15). M7-1: 8.4% (day 56); 4.5% (day 117)

Bentazone rapidly decreased from 95.6% of the applied radioactivity on day 0 to 37.8% of the applied radioactivity after 14 days of incubation. Thereafter it further decreased to 1.3% of the applied radioactivity after 117 days. Results indicated, that ^{14}C-bentazone was degraded to at least eight minor radioactive fractions, none of them exceeding individually 2.1% of AR. The radioactivity extracted by harsh extraction (M7) was shown to consist of at least four radioactive fractions by TLC analysis.

Calculated DT50 and DT90 values obtained by applying first-orderkinetics are 9.6 days (DT_{50}) and 32 days (DT_{90}). In figure B.8.1.1-02 the fitted parameters are reported.

Figure B.8.1.1-02 First order kinetic fit for bentazone degradation



Conclusion

^{14}C-bentazone degraded rapidly in the soil tested with calculated DT_{50} and DT_{90} values of 9.6 days and 32 days, respectively. Up to eight minor degradation products were detected. None of them individually exceeded 2.8% of the applied radioactivity. The amount of ^{14}CO2 amounted up to 14.9% of AR whereas the amount of other volatiles was insignificant, not exceeding 0.1%. Non-extractable radioactivity, was contributing 53.5% of AR after 117 days.

Comments

The study was conducted according to guidelines preceding current guidelines. The study can nevertheless still be considered acceptable for persistence endpoints as the measurements and results are in line with current guidelines. A re-analysis of the study results is provided in the paragraph on rate of degradation B.8.1.2.

B.8.1.1.2 Supplementary studies (Annex IIA 7.1.2 to 7.1.3)

Anaerobic degradation

Main notifier

STUDY IIA, 7.1.2/01

Characteristics

reference	:	Ebert, D. 2010b	study type	:	anaerobic degradation
year of execution	:	2010	incubation time	:	up to 120 d
GLP statement	:	yes	nominal concentration	:	2.4 mg/kg
guideline	:	Incl. OECD 307 (2002)	temperature	:	20°C
test substance	:	Bentazone Lablled: phenyl-U-^{14}C, Batch 2010-2301 Unlabelled: batch 01893-210	DT50	:	see results
purity	:	chemical purity 99.8%, radiochemical purity 97.7%.	metabolites	:	see results
soil	:	sandy loam	acceptability	:	**acceptable**
			Previous evaluation	:	Submitted for renewal (supplementary)

Study design

The anaerobic soil metabolism of ^{14}C-bentazone (BAS 351 H) was investigated in a German soil (Bruch West, Limburgerhof) collected freshly from the field. Soil characteristics are reported in table B.8.1.2-01 below.

Table B.8.1.1.2-01 Properties of soil Bruch West used to investigate the degradation rate of ^{14}C-Bentazone under anaerobic conditions

Soil designation	**Bruch West (09/060/02)**
Origin	Limburgerhof, RP, Germany
USDA Particle size distribution [%]	
sand 0.050 – 2 mm	61.0
silt 0.002 – 0.050 mm	27.1
clay < 0.002 mm	11.9
textural class	sandy loam

Organic C [%] Organic matter [%] *	1.60 2.76
pH [H_2O] pH [$CaCl_2$]	7.9 7.1
Cation exchange capacity [$cmol^+$ / kg]	12.5
Maximum water holding capacity [g/100g dry soil]	29.3
Microbial biomass [mg C/100g dry soil]	38.7**

*organic matter = organic carbon x 1.724

**optimal glucose: 0.4%

Soil was treated at a concentration of about 2.4 mg bentazone/kg dry soil, and adjusted to 40% of MWHC. Soils were incubated for 14 days under aerobic conditions in the dark at a temperature of 20 ± 1°C. Then the half-life of bentazone in soil was almost reached and the soil was flooded with water (day 15). The aeration was switched to nitrogen in order to establish anaerobic conditions for the remaining incubation period. All exiting air/nitrogen was passed through a trapping system consisting of flasks containing ethylene glycole and aqueous sodium hydroxide for trapping organic volatiles and $^{14}CO_2$, respectively. Samples were taken at 0, 3, 7, 14, 21, 28, 42, 77 and 120 days after treatment (DAT). Soil samples were extracted with methanol and methanol/water (1:1) mixtures . The extracts were measured for radioactivity by LSC, then combined, concentrated and subjected to radio-HPLC analysis. The non-extractable residues were determined by combustion and subsequent LSC measurement. The evolved $^{14}CO_2$ from each aliquot was trapped in Oxysolve C-400 scintillator and measured by LSC.The radioactivity in the volatile trapping solutions was also determined by LSC. A total balance of radioactivity was established for each sampling interval. The procedural recoveries during the work-up (i.e. after concentration of the extracts for HPLC analysis) were checked routinely by LSC. Recoveries were always ≥ 99 %.

Results

During the aerobic incubation phase, the amount of extractable radioactivity decreased rapidly from 100 %TAR at day 0 to 57 %TAR at day 14. After establishing anaerobic conditions from day 15 onwards, no significant further decline of extractability was observed. The extractable residues remained in the range of 53 – 57 %TAR until 120 days. No metabolite exceeded 1 %TAR at any sampling time. By comparison of the retention time with certified reference item, the peak eluting at 39 min could be assigned to the known soil metabolite N-methyl-bentazone (BH 351-N-Me). However, its amounts never exceeded 0.5 %TAR.

The non-extractable ^{14}C-residues increased to 39 %TAR within the first 14 days under aerobic incubation and then remained at a rather constant level of 40 - 43 %TAR under anaerobic conditions. Formation of CO_2

(mineralization) increased to about 5 %TAR during the first two weeks of aerobic incubation and then quickly slowed down under anaerobic conditions, reaching finally 6.5 % TAR after 120 days. No other volatiles were detected. The material balance was always in the range between 100.1 and 102.7% TAR. A summar of the results is presented in table B.8.1.2-02 below.

Table B.8.1.1.2-02 Distribution of radioactivity in soil after treatment with ^{14}C-bentazone following 14 days of aerobic and 106 days of anaerobic incubation [% TAR]

Days after treatment	**Extractable**					**NER**	**Volatiles**		**Material balance**
	Methanol 1	**Methanol 2**	**Methanol/ Water 1**	**Methanol/ Water 2**	**Total extractable**		**Ethylene glycol**	**CO_2 (NaOH)**	
Aerobic pre-incubation									
0	64.7	21.5	11.2	3.3	100.7	1.9	-	-	102.7
0	63.3	22.0	11.0	3.2	99.5	2.0	-	-	101.5
0 mean	64.0	21.8	11.1	3.3	100.1	2.0	-	-	102.1
3	57.6	16.1	9.4	2.6	85.7	14.1	0.0	0.8	100.6
7	47.4	15.2	9.1	2.7	74.5	23.5	0.0	2.1	100.1
14	37.1	11.1	7.0	2.2	57.4	39.3	0.0	4.7	101.4
Days after treatment	**Extractable**						**Volatiles**		**Material balance**
	Methanol/Water 1	**Methanol/ Water 2**	**Methanol 1**	**Methanol 2**	**Total extractable**	**NER**	**Ethylene glycol**	**CO_2 (NaOH)**	
Anaerobic incubation									
21	43.9	6.5	1.9	0.6	52.9	43.2	0.0	5.3	101.4
28	46.5	7.1	2.3	0.7	56.6	39.5	0.0	5.4	101.5
42	46.4	6.8	1.9	0.5	55.7	39.6	0.0	5.6	100.9
42	44.3	6.0	1.8	0.4	52.5	43.2	0.0	5.6	101.3
42 mean	45.4	6.4	1.9	0.5	54.1	41.4	0.0	5.6	101.1

77	47.4	6.6	1.9	0.6	56.5	40.0	0.0	6.1	102.6
120	44.6	6.7	2.4	0.7	54.4	40.0	0.0	6.5	101.0
120	43.6	7.4	2.1	0.6	53.7	41.0	0.0	6.5	101.2
120 mean	44.1	7.0	2.3	0.7	54.1	40.5	0.0	6.5	101.1

Conclusion

The DT_{50} for the aerobic incubation was calculated to be 18.5 days (best fit, FOMC), whereas for the anaerobic phase no degradation was observed (DT_{50} > 1000 days).

Comment

The study is acceptable. The result that bentazone does not degrade under anaerobic conditions is considered for risk assessment.

B.8.1.2 Rate of degradation in soil (Annex IIA 7.2; Annex IIIA 9.1)

B.8.1.2.1 Laboratory studies Rate of degradation at 20ºC; (Annex IIA 7.2.1; Annex IIIA 9.1)

Main notifier data/data from original DAR

parent substance

reference	:	Anonymous, BOD96-00058	GLP statement	:	no
Type of study	:	Rate of degradation in soil	Guideline	:	BBA merkblatt 36
Year of execution	:	1974	Acceptability	:	Not acceptable
test substance	:	Bentazone, not specified	Previous evaluation	:	In original DAR : acceptable

Method:

Determination of degradation rates of unlabeled bentazone according BBA Merkblatt 36.

Soil characteristics:

soiltype	Clay [%]	Silt [%]	Sand [%]	org.C [%]	pH	MWHC [%]	CEC [meq/100g]
Loamy	10.1 (<20µ)			2.6	6.8	n.r.	n.r.

sand					
Loamy sand	19.5 (<20µ)	1.0	5.2	n.r.	n.r.

Results :

Recoveries: 80 % (soil 9) and 100 % (soil 10). Further results see Summary.

Comment:

Acceptable.

RMS comment 2013: The study was considered acceptable in the original DAR however, based on the reduced information vailable in the original report and the short summary presented in the original DAR RMS cannot confirm this study can still be considered acceptable, even when the requirements with regard to study quality at the time of the original DAR are taken in consideration.

reference	:	Keller, BOD96-124	GLP statement	:	no
Type of study	:	Rate of degradation in soil	Guideline	:	BBA Guideline IV, 4-1
Year of execution	:	1988	Acceptability	:	Not acceptable
test substance	:	Bentazone, not specified	Previous evaluation	:	In original DAR : acceptable

Method:

In laboratory experiments the degradation behaviour of bentazone was examined in four soils. The study was carried out according to BBA Guideline IV, 4-1 from December 1986. 0, 2, 4, 8, 16, 32, 64 and 100 days after application duplicate samples were taken from the degradation study and analyzed or deep frozen at -2 0°C. The samples were analyzed by methylation with iodomethane in the presence of tetrabutylammonium hydrogen sulfate. Clean up on Florisil column and GC-determination of the N-methyl-bentazone derivative was conducted using the S-FPD.

Soil characteristics:

soiltype	Clay	Silt	Sand	org.C	pH	MWHC	CEC	Microbial biomass [mg

	[%]	[%]	[%]	[%]		[%]	[meq/100g]	C/100 g	
								start	end
Loam	38.6 (<20µm)			1.4	7.2	49	14.8	24.5	24.5
Loamy sand	18.0 (<20µm)			0.5	6.7	40	5.2	16.8	10.9
Sandy loam	21.0 (<20µm)			2.4	7.1	36	10.4	50.5	49.9
Loamy sand	12.6 (20µm)			2.3	5.8	44	2.4	32.8	24.8

Results: See Summary.

Comment:

Acceptable.

RMS comment 2013: The studies were considered acceptable in the original DAR however, based on the reduced information vailable in the original report and the short summary presented in the original DAR RMS cannot confirm this study can still be considered acceptable, even when the requirements with regard to study quality at the time of the original DAR are taken in consideration

A summary of already peer-reviewed DT50 values from the previous Annex I evaluation as listed in the EU review report (2000) is given below. The degradation rates of bentazone under laboratory conditions showed a rather high variability. DT_{50} values ranged from 8 to 102 d with an average of 45 days according to the EU review report. In the "Monograph of Bentazone, 17 Sept 1996", the basis of the laboratory rate of degradation endpoints is given in the summary table shown in Table B.8.2.1/01.

Table B.8.1.2.1-01 Summary of rate of degradation of bentazone from original DAR

Summary: Rate of degradation in laboratory studies

Calculation of DT50 and DT90 values (days)*) of bentazone :

Soil no.	appl. rate mg/kg	pH	Moisture %	Temperature °C	$DT50_l$ d	$DT90_l$ d	Ref.
1	2	6.7	15	22 +/- 2	12	39	(1)
1	5	6.7	15	22 +/- 2	25	-	(1)
Influence of Moisture							
1	2	6.7	5	22 +/- 2	20	66	(1)
1	2	6.7	10	22 +/- 2	18	59	(1)
1	2	6.7	15	22 +/- 2	23	76	(1)
Influence of Temperature							
1	2	6.7	12	8-10	161**)	-	(1)
1	2	6.7	12	22+/- 2	35**)	-	(1)
1	2	6.7	12	35-37	35**)	-	(1)
Influence of pH							
4	2	6.4	12	22 +/- 2	34	113	(1)
4	2	4.6/5.5	12	22 +/- 2	10	33	(1)
2	2	7.5	20	22 +/- 2	56	-	(1)
2	3/3	7.5	20	22 +/- 2	70/84	-	(3)
3	2	5.1	20	22 +/- 2	102	-	(1)
4	3/3/3	4.6	12	22 +/- 2	42/46/47	-	(2)
5	10	6.1	52	22 +/- 2	65	215	(4)
6	10	5.0	31	22 +/- 2	45	151	(4)
7	10	7.7	61	22 +/- 2	45	150	(4)
9	5	6.8	40	20	38	125	(5)
10	5	5.2	40	20	16	54	(5)
11	2	7.2	40	20	11	37	(6)
12	2	6.7	40	20	47	198(extr.)	(6)
13	2	7.1	40	20	80	-	(6)
14	2	5.8	40	20	8	85	(6)

*) All calculation were conducted according (7)(8)
-if not stated otherwise.
**) Graphical interpolation

The basis of the studies and the selection of mean parameters are the following references:

1. Drescher and Otto (1972). Studies on the degradation of bentazone (BAS 351-H) in soil. BASF DocID 1972/0030.
2. Drescher and Otto (1973). Studies on the degradation of bentazone (BAS 351-H) in soil – 2nd report. BASF DocID 1973/0031.
3. Drescher and Otto (1973). Über den Abbau von Bentazon im Boden (4. Mitteilung). BASF DocID 1973/0047.

4. Anonym (1974). Verhalten des Pflanzenschutzmittelwirkstoffes im Boden. BASF DocID 1974/10086.
5. Keller, E. (1987). The aerobic soil metabolism of BAS 351 H (Bentazone). BASF DocID 1987/0415.
6. Keller, W. (1988). Degradation behavior of Bentazone in soil. BASF DocID 1988/10121.

Applicant 1, main notifier of the original dossier stated that the studies that are mentioned in the original Monograph were not carried out according to current OECD guideline (OECD, 307). Old analytical methods were used and for some studies the measured concentrations were not even given in the report but only shown as graphs. RMS would like to add that from the information reported in the DAR sufficient information to verify the endpoints for degradation cannot be derived. Furthermore, from the original DAR summaries it can be read that the study with reference 6 in the table above was considered not acceptable.

For the renewal dossier a new rate of degradation study was initiated to cover for the mentioned deficiencies.

Under chapter B.8.1.1 study 7.1.1/06 on the route of degradation was discussed and reported. Also the rate of degradation from that study is calculated and reported.

STUDY IIA, 7.2.1/01

Characteristics

reference	:	Tornisielo A., Sacchi R.R. 2011(b)	study type	:	aerobic degradation
year of execution	:	2010-2011	incubation time	:	up to 120 d
GLP statement	:	Yes	nominal concentration	:	2 mg/kg
guideline	:	OECD 307 (2002)	temperature	:	20°C
test substance	:	U-^{14}C bentazone, batch no. 210-2201.	DT50	:	see results
purity	:	chemical purity not reported, radiochemical purity 99.1%	metabolites	:	acceptable
soils	:	loamy sand (2x) and sandy loam (2x)	acceptability	:	**acceptable**
			Previous evaluation	:	Submitted for

					renewal (essential)

Study design

The degradation rate of BAS 351 H (bentazone) under aerobic conditions was investigated in four different soils at a temperature of 20°C. The soils were typical agricultural soils from Germany, freshly collected from the field. Soil properties are reported in table B.8.2.1-02.

The soils were treated with a nominal rate of 2.0 mg ^{14}C-labeled BAS 351 H per kg dry soil which corresponds to a field application rate of 750 g bentazone/ha, assuming equal distribution in the upper 2.5 cm soil layer and a soil density of 1.5 g/cm^3. Incubations were in the dark at soil moisture of 40 % of the maximum water holding capacity. A closed incubation system with continuous aeration (moistened air) was used with an attached trapping system for the determination of volatile compounds. Samples were taken at 0, 3, 7, 14, 29, 62, 90, and 120 days after treatment (DAT).

Table B.8.1.2.1-02 Properties of soils used to investigate degradation rate of bentazone

Soil designation	**Bruch West (09/060/01)**	**Li 10 (09/1680/01)**	**Lufa 2.2 (09/736/01)**	**Lufa 2.3 (09/570/01)**
Origin	Limburgerhof, RP, Germany	Limburgerhof, RP, Germany	Hanhofen, RP, Germany	Offenbach, RP, Germany
USDA Particle size distribution [%]	63.7	80.2	85.6	77.1
sand 0.050 – 2 mm	24.6	14.3	9.6	16.5
silt 0.002 – 0.050 mm	11.7	5.6	4.8	6.4
clay < 0.002 mm	sandy loam	loamy sand	loamy sand	sandy loam
textural class				
Organic C [%]	1.37	0.97	0.15	0.98
Organic matter [%] *	2.36	1.67	0.26	1.69
pH [H_2O]	7.8	6.8	6.2	7.4
pH [$CaCl_2$]	7.1	6.1	5.7	6.7
Cation exchange capacity [cmol$^+$ / kg]	10.1	4.7	5.5	7.2

Maximum water holding capacity [g/100g dry soil]	28.2	25.7	33.8	25.0
Microbial biomass - before start of study [mg C/100g dry soil]	37.0**	19.4**	34.1**	19.4**
Microbial biomass - 62 DAT [mg C/100g dry soil]***	34	24	26	19
Microbial biomass - 120 DAT [mg C/100g dry soil]***	41	27	27	34

* organic matter = organic carbon x 1.724

** optimal glucose: 0.2%

*** determined at test facility

RP Rhineland-Palatinate

After sampling the soil samples were extracted twice with methanol and four times with methanol/water (1:1) and the extracts were concentrated and analysed by means of liquid scintillation counting (LSC) and HPLC. The amount of non-extractable residues was determined by combustion and subsequent LSC measurements. The microbial biomass of the soils was determined after 62 and 120 days of incubation according to the method by Anderson & Domsch.

Kinetic analysis and calculations of DT_{50} and DT_{90} values for bentazone were performed following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006).* The analysis was conducted by non-linear regression methods employing the software tool KinGUl 1.1, applying single first order kinetics (SFO) and first order multi compartment kinetics (FOMC). The goodness-of-fit was evaluated by visual assessment, χ^2 minimum error and type-I-error rate (t-test). For calculations, the measured values at DAT 0 and all later sampling time points (1, 3, 7, 14, 29, 62, 90 and 120) were set to the amount of extractable residues. Replicate measurements where available were used for parameter estimation.

Results

The distribution of radioactive residues in the different soils treated with ^{14}C-labeled bentazone at various time intervals from 0 DAT to 120 DAT is shown in Table B.8.2.1/03 to Table B.8.2.1/06. The material balance ranged from 91.8% to 111.8% TAR for all soils and samples with average values of 98.6% (Bruch West), 98.3% (Li10) and 100.3% (LUFA 2.2) and 98.6% (LUFA 2.3). Extractable radioactivity decreased from 96.4-100.9 % TAR at 0 DAT to about 6.9-19.4 % TAR after 120 days. The predominant part of the

radioactivity could be extracted with methanol (91.3 to 3.1 % TAR). Subsequent extraction with methanol/water yielded additional 3.8 to 9.6 % TAR. The acetone fractions obtained by rinsing the soil with acetone after the last extraction step contained only negligible amounts of radioactivity (≤0.8% TAR).

Non-extractable radioactive residues in all four soils were formed in high amounts reaching their maximum of 63.8 to 92.5 % TAR at the end of the study after 120 days of incubation.

$^{14}CO_2$ was observed in all four soils reaching in total 9.1 to 21.2 % TAR after 120 days. No other volatile compounds were detected.

Table B.8.1.2.1-03 Distribution of radioactivity in soils after treatment with ^{14}C-bentazone and incubation under aerobic conditions [% TAR] – Bruch West (20°C)

Days after treatment	Extractable				NER	Volatiles*			Material balance
	Methanol	Methanol + water	Acetone	Total		CO_2	Other volatiles	Total	
0	87.5	9.6	0.1	97.2	2.9	n.d.	n.d.	n.d.	100.1
0	87.5	9.5	0.0	97.0	2.9	n.d.	n.d.	n.d.	99.9
0 mean	87.5	9.6	0.1	97.1	2.9	n.d.	n.d.	n.d.	100.0
3	81.2	9.2	0.2	90.6	9.8	0.0	0.0	0.0	100.4
7	72.3	8.6	0.4	81.2	17.7	0.0	0.0	0.0	98.9
14	62.4	7.9	0.1	70.5	27.5	1.4	0.0	1.4	99.4
29	44.8	7.6	0.1	52.5	41.3	5.7	0.0	5.7	99.5
62	21.1	6.5	0.3	27.8	58.2	13.0	0.0	13.0	98.9
62	21.0	6.4	0.3	27.6	58.2	13.9	0.0	13.9	99.8
62 mean	21.0	6.4	0.3	27.7	58.2	13.5	0.0	13.5	99.3
90	8.6	4.3	0.1	13.1	62.8	17.9	0.0	17.9	93.8
120	3.0	3.8	0.1	6.9	69.2	21.2	0.0	21.2	97.4
120	3.1	4.1	0.1	7.3	67.8	21.2	0.0	21.2	96.3
120 mean	3.1	4.0	0.1	7.1	68.5	21.2	0.0	21.2	96.8

n.d. = not determined

NER = non-extractable residues

TAR = total applied radioactivity (100% = 1.949 mg/kg dry weight)

*cumulated values

Table B.8.1.2.1-04 Distribution of radioactivity in soils after treatment with ^{14}C-bentazone and incubation under aerobic conditions [% TAR] –Li10 (20°C)

Days after treatment	Extractable				NER	Volatiles*			Material balance
	Methanol	Methanol + water	Acetone	Total		CO_2	Other volatiles	Total	
0	90.8	7.6	0.0	98.5	2.0	n.d.	n.d.	n.d.	100.4
0	90.1	6.9	0.1	97.0	2.5	n.d.	n.d.	n.d.	99.6
0 mean	90.4	7.3	0.1	97.8	2.2	n.d.	n.d.	n.d.	100.0
3	88.3	5.2	0.1	93.6	6.4	0.0	0.0	0.0	100.0
7	79.9	5.8	0.3	86.0	11.5	0.0	0.0	0.0	97.5
14	69.4	6.5	0.1	76.0	22.8	0.6	0.0	0.6	99.4
29	53.2	7.1	0.1	60.4	29.2	2.5	0.0	2.5	92.1
62	28.0	6.1	0.2	34.2	63.0	6.5	0.0	6.5	103.7
62	30.1	6.5	0.2	36.8	58.0	6.8	0.0	6.8	101.5
62 mean	29.1	6.3	0.2	35.5	60.5	6.6	0.0	6.7	102.6
90	18.6	5.5	0.1	24.2	61.0	10.2	0.0	10.2	95.4
120	10.0	5.3	0.3	15.6	67.4	13.5	0.0	13.5	96.5
120	10.9	4.0	0.1	15.0	63.8	13.5	0.0	13.5	92.3
120 mean	10.4	4.6	0.2	15.3	65.6	13.5	0.0	13.5	94.4

n.d. = not determined

NER = non-extractable residues

TAR = total applied radioactivity (100% = 1.958 mg/kg dry weight)

*cumulated values

Table B.8.1.2.1-05 Distribution of radioactivity in soils after treatment with ^{14}C-bentazone and incubation under aerobic conditions [% TAR] – LUFA 2.2 (20°C)

Days after treatment	Extractable				NER	Volatiles*			Material balance
	Methanol	Methanol + water	Acetone	Total		CO_2	Other volatiles	Total	
0	84.7	9.1	0.1	93.8	2.8	n.d.	n.d.	n.d.	96.6
0	91.3	9.6	0.1	100.9	2.4	n.d.	n.d.	n.d.	103.4
0 mean	88.0	9.4	0.1	97.4	2.6	n.d.	n.d.	n.d.	100.0
3	73.3	9.0	0.3	82.6	14.6	0.0	0.0	0.0	97.2
7	69.1	8.4	0.1	77.6	18.2	0.0	0.0	0.0	95.9
14	54.7	8.3	0.2	63.2	35.8	0.6	0.0	0.6	99.6
29	45.0	8.9	0.5	54.4	39.9	2.6	0.0	2.6	96.9
62	14.3	6.0	0.3	20.6	67.5	4.7	0.0	4.7	92.8
62	13.1	5.9	0.3	19.3	67.0	5.5	0.0	5.5	91.8
62 mean	13.7	5.9	0.3	19.9	67.2	5.1	0.0	5.1	92.3
90	8.3	4.8	0.2	13.4	81.8	7.4	0.0	7.4	102.6
120	6.5	4.9	0.3	11.7	92.5	9.1	0.0	9.1	113.2
120	6.3	4.6	0.3	11.3	90.0	9.1	0.0	9.1	110.4
120 mean	6.4	4.8	0.3	11.5	91.3	9.1	0.0	9.1	111.8

n.d. = not determined

NER = non-extractable residues

TAR = total applied radioactivity (100% = 1.891 mg/kg dry weight)

*cumulated values

Table B.8.1.2.1-06 Distribution of radioactivity in soils after treatment with ^{14}C-bentazone and incubation under aerobic conditions [% TAR] – LUFA 2.3 (20°C)

Days after treatment	Extractable				NER	Volatiles*			Material balance
	Methanol	Methanol + water	Acetone	Total		CO_2	Other volatiles	Total	
0	86.9	9.3	0.2	96.4	2.5	n.d.	n.d.	n.d.	98.9
0	89.6	8.7	0.4	98.6	2.5	n.d.	n.d.	n.d.	101.1
0 mean	88.2	9.0	0.3	97.5	2.5	n.d.	n.d.	n.d.	100.0
3	81.9	7.8	0.3	90.0	8.1	0.0	0.0	0.0	98.1
7	76.5	8.1	0.2	84.8	12.5	0.0	0.0	0.0	97.3
14	65.8	8.2	0.2	74.1	21.2	0.7	0.0	0.7	96.0
29	51.9	9.5	0.8	62.2	31.2	3.2	0.0	3.2	96.6
62	34.8	8.9	0.7	44.3	49.6	8.9	0.0	8.9	102.9
62	29.8	7.5	0.3	37.5	51.0	7.8	0.0	7.8	96.2
62 mean	32.3	8.2	0.5	40.9	50.3	8.3	0.0	8.4	99.5
90	19.1	6.4	0.2	25.8	58.0	12.6	0.0	12.6	96.3
120	13.1	6.1	0.2	19.4	62.8	16.0	0.0	16.0	98.2
120	11.7	5.1	0.1	16.9	67.7	16.0	0.0	16.0	100.6
120 mean	12.4	5.6	0.2	18.1	65.2	16.0	0.0	16.0	99.4

n.d. = not determined

NER = non-extractable residues

TAR = total applied radioactivity (100% = 1.954 mg/kg dry weight)

*cumulated values

All combined methanol and methanol/water extracts were analysed by radio-HPLC. The results are summarized in Table B.8.2.1/07 to Table B.8.2.1/10. The concentration of the parent compound decreased in the four soils from 92.6 % TAR at 0 DAT to approximately 4.8-18.8 % TAR after 120 days

The metabolite N-methyl-bentazone (M351H009) was detected in three soils, however only in small amounts showing maximum values of 2.2 (Li 10), 5.4 (LUFA 2.2) and 0.5 (LUFA 2.3) % TAR.

Two minor unknown metabolites were visible in the chromatograms showing maximum values of 2.2 (UK2) and 1.5 (UK1) % TAR.

Table B.8.1.2.1-07 Radio-HPLC analysis of soil extracts after treatment of soil Bruch West with ^{14}C-bentazone and incubation under aerobic conditions at 20°C [%TAR]

Days after treatment	**Total**	**UK1**	**Bentazone (BAS 351 H)**	**N-methyl-bentazone (M351H009)**	**UK2**
		t_R 30.9	**t_R 37.9**	**t_R 47.6**	**t_R 51.6**
0	97.2	0.7	96.5	-	-
0	97.0	1.0	96.1	-	-
0 mean	97.1	0.8	96.3	-	-
3	90.6	0.4	90.2	-	-
7	81.2	0.7	80.5	-	-
14	70.5	1.2	69.2	-	-
29	52.5	0.9	51.6	-	-
62	27.8	-	25.9	-	1.9
62	27.6	-	27.6	-	-
62 mean	27.7	-	26.8	-	0.9
90	13.1	-	13.1	-	-
120	6.9	-	6.9	-	-
120	7.3	-	7.3	-	-
120 mean	7.1	-	7.1	-	-

t_R = retention time [min]

- = not detected

TAR = total applied radioactivity (100% = 1.949 mg/kg dry weight)

Table B.8.1.2.1-08 Radio-HPLC analysis of soil extracts after treatment of soil Li10 with ^{14}C-bentazone and incubation under aerobic conditions at 20°C [%TAR]

Days after treatment	Total	UK1	Bentazone (BAS 351 H)	N-methyl-bentazone (M351H009)	UK2
		t_R 30.9	t_R 37.9	t_R 9.5	t_R 51.6
0	98.5	0.5	98.0	-	-
0	97.0	-	96.8	-	0.2
0 mean	97.8	0.2	97.4	-	0.1
3	93.6	1.0	92.6	-	-
7	86.0	-	85.8	-	0.2
14	76.0	1.5	74.5	-	-
29	60.4	1.4	59.1	-	-
62	34.2	-	34.2	-	-
62	36.8	-	36.0	0.8	-
62 mean	35.5	-	35.1	0.4	-
90	24.2	-	21.4	2.3	0.5
120	15.6	-	13.4	2.2	-
120	15.0	-	13.3	1.7	-
120 mean	15.3	-	13.3	2.0	-

tR = retention time [min]

- = not detected

TAR = total applied radioactivity (100% = 1.958 mg/kg dry weight)

Table B.8.1.2.1-09 Radio-HPLC analysis of soil extracts after treatment of soil LUFA 2.2 with ^{14}C-bentazone and incubation under aerobic conditions at 20°C [%TAR]

Days after treatment	Total	UK1	Bentazone (BAS 351 H)	N-methyl-bentazone (M351H009)	UK2
		t_R 30.9	t_R 37.9	t_R 9.5	t_R 51.6
0	93.8	1.2	92.6	-	-
0	100.9	1.0	100.0	-	-
0 mean	97.4	1.1	96.3	-	-
3	82.6	0.6	81.9	-	-
7	77.6	0.4	77.0	-	0.3
14	63.2	0.5	62.7	-	-
29	54.4	-	50.5	1.8	2.2
62	20.6	-	17.1	3.5	-
62	19.3	-	16.1	3.2	-
62 mean	19.9	-	16.6	3.3	-
90	13.4	-	8.7	4.7	-
120	11.7	-	6.7	4.1	0.8
120	11.3	-	4.8	5.4	1.0
120 mean	11.5	-	5.8	4.8	0.9

tR = retention time [min]

- = not detected

TAR = total applied radioactivity (100% = 1.891 mg/kg dry weight)

Table B.8.1.2.1-10 Radio-HPLC analysis of soil extracts after treatment of soil LUFA 2.3 with ^{14}C-bentazone and incubation under aerobic conditions at 20°C [%TAR]

Days after treatment	Total	UK1	Bentazone (BAS 351 H)	N-methyl-bentazone (M351H009)	UK2
		t_R 30.9	t_R 37.9	t_R 9.5	t_R 51.6

0	96.4	-	95.0	-	1.4
0	98.6	-	96.6	-	2.0
0 mean	97.5	-	95.8	-	1.7
3	90.0	0.7	89.3	-	-
7	84.8	1.0	83.8	-	-
14	74.1	0.5	73.6	-	-
29	62.2	1.3	59.7	-	1.3
62	44.3	-	44.3	-	-
62	37.5	-	37.0	0.3	0.2
62 mean	40.9	-	40.6	0.2	0.1
90	25.8	0.3	25.2	0.2	-
120	19.4	-	18.8	0.5	-
120	16.9	-	16.6	0.3	-
120 mean	18.1	-	17.7	0.4	-

tR = retention time [min]

- = not detected

TAR = total applied radioactivity (100% = 1.954 mg/kg dry weight)

An overview on the dissipation times of bentazone calculated with the different kinetics is given in Table B.8.2.1/11 and B.8.2.1/12.

The χ^2 error level was in all cases lower than 15%, indicating accurate statistical description by the applied kinetic models. The SFO model showed a better fit for the Bruch West and the LUFA 2.2 soil whereas the FOMC fit was slightly better for the Li10 and LUFA 2.3 fit. However, the fitted parameters were not significant on a 5% level for the FOMC fit of LUFA 2.3.

For all fits, the goodness of the fit was confirmed by visual inspection. The residuals are distributed randomly. Due to the very low χ^2 error level and the higher significance of the SFO kinetic parameters, the SFO kinetics can be regarded as persistence endpoints as well as modelling endpoints in all cases with the exception of the Li 10 soil where the FOMC fit should be used to obtain persistence endpoints.

Table B.8.1.2.1-11 DT_{50}/DT_{90} values for bentazone in various soils at 20°C calculated with the SFO model]

Soil	**DT_{50} [days]**	**DT_{90} [days]**	**Type I error rate (Prob.>t)**	**χ^2 error level**	**Visual fit**
Bruch West	**33.0**	**109.6**	**9.1×10^{-13}**	**1.77**	**excellent**
Li10	43.4	144.2	5.4×10^{-13}	1.38	excellent
LUFA 2.2	**30.9**	**102.7**	**2.5×10^{-7}**	**6.65**	**excellent**
LUFA 2.3	**49.1**	**163.2**	**4.9×10^{-10}**	**2.52**	**excellent**

Table B.8.1.2.1-12 DT_{50}/DT_{90} values for bentazone in various soils at 20°C calculated with the FOMC model]

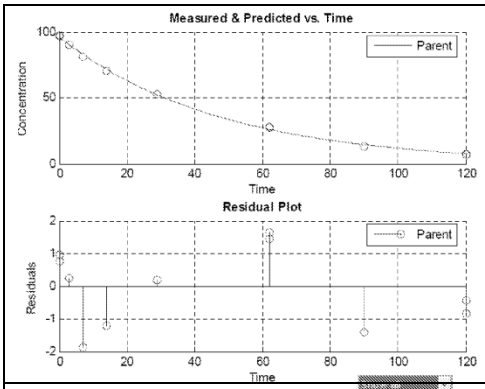
Soil	**DT_{50} [days]**	**DT_{90} [days]**	**χ^2 error level**	**α and β at 5% error level**	**Visual fit**
Bruch West	32.1	112.6	2.00	not significant	excellent
Li10	**42.0**	**152.7**	**0.88**	**significant**	**excellent**
LUFA 2.2	27.4	119.0	6.78	not significant	excellent
LUFA 2.3	46.0	188.9	2.36	not significant	excellent

best fit values printed in bold

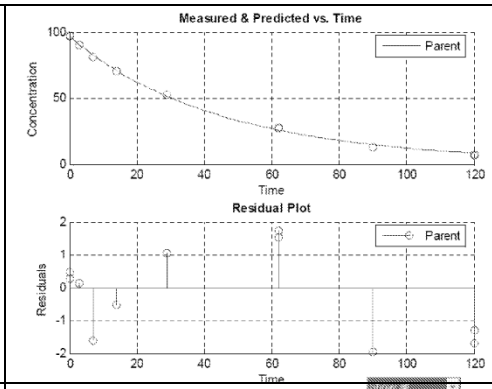
The fitted curves for the single first-order (SFO) and the first-order multiple compartments (FOMC) are shown in the figures below.

figure B.8.1.2.1-01 ^{14}C-bentazone: modelled against observed residue

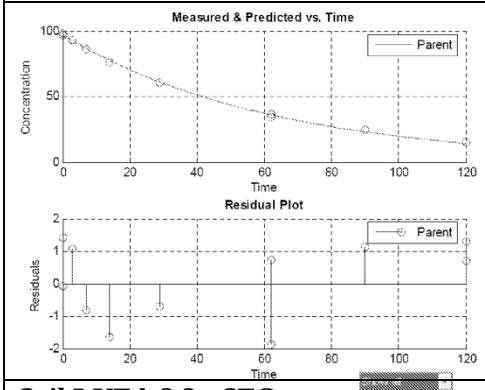
Bruch West - SFO	**Bruch West - FOMC**



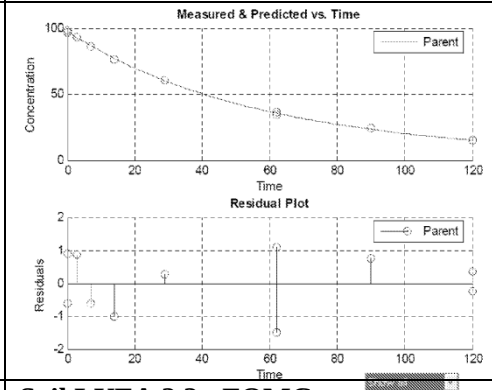
Soil Li 10 - SFO



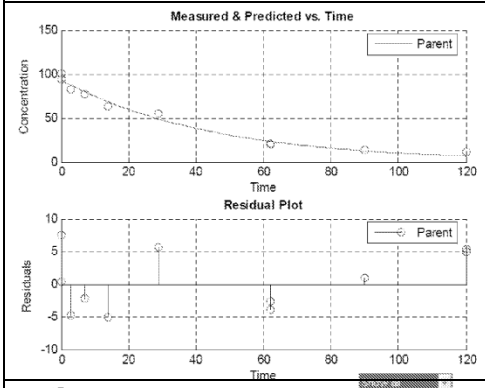
Soil Li 10 - FOMC



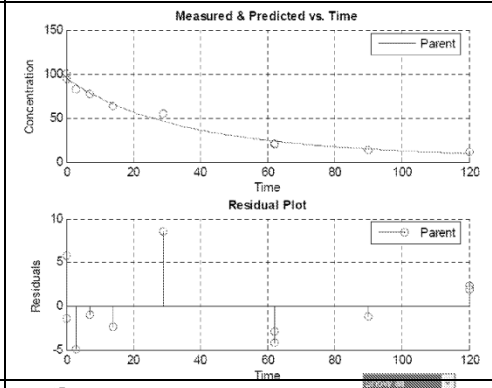
Soil LUFA 2.2 - SFO



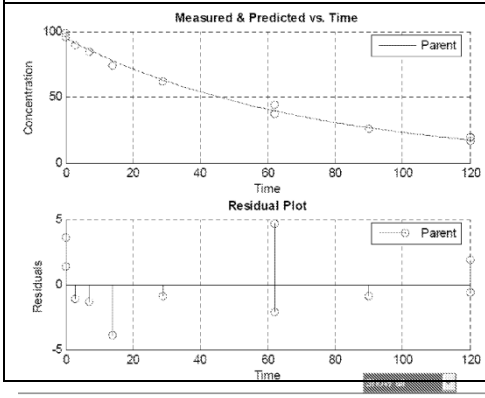
Soil LUFA 2.2 - FOMC

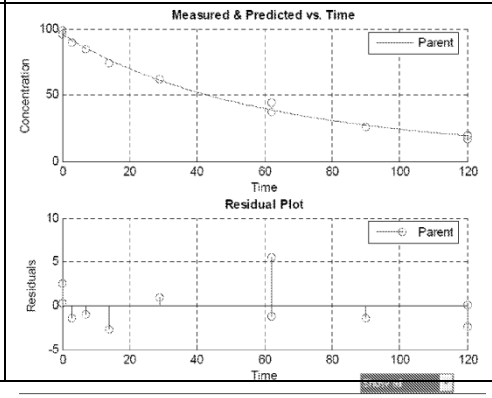


Soil LUFA 2.3 - SFO



Soil LUFA 2.3 - FOMC





Conclusion

The results of the present study show that bentazone was degraded in four different soils with SFO half-lives of 33.0 (Bruch West), 43.4 (Li 10), 30.9 (LUFA 2.2) and 49.1 days (LUFA 2.3) when incubated under aerobic conditions at 20°C and a soil moisture of 40 % of the maximum water holding capacity.

In three soils, the known soil metabolite N-methyl-bentazone was detected at a maximum amount of 5.4% TAR (LUFA 2.2). Two minor unknown metabolites appeared during the course of the study; however, none of them exceeded 2.2 % of the total applied radioactivity. Mineralization rate reached 9.1-21.2% TAR at the end of the study and the non-extractable residues amounted to 65.2 - 91.3% TAR.

B.8.1.2.2 Laboratory studies rate of degradation at 10ºC (Annex IIA 7.2.1; Annex IIIA 9.1)

parent substance

Aerobic degradation half-lives at 10 °C can be derived by extrapolation from degradation half-lives obtained from e.g. 20°C-data using a Q10-value of 2.58 as proposed by EFSA, 2007 (Opinion on the default Q10 value).

Therefore the half-lives of the degradation rate study with bentazone described in Section II A 7.2.1/1 (Tornisielo & Sacchi, BASF DocID 2011/1000621) were extrapolated to 10 °C according to the following equation.

$$f_{temp} = Q_{10}^{\left(\frac{Temp_{act} - Temp_{ref}}{10}\right)}$$

where:

f_{temp} = temperature normalisation factor

Q10 = factor of change of the degradation rate at temperature change of 10°C (2.58)

$Temp_{ref}$ = reference temperature to which the degradation rate is scaled [here: 10 °C]

$Temp_{act}$ = temperature at which the study was carried out [here: 20 °C]

The extrapolated half-lives are shown in Table 7.2/17. The resulting DegT50 values scaled to 10 °C range from 79.7 to 126.7 days with a geometric mean value of 99.0 days. The DegT90 values scaled to 10 °C range from 264.8 to 420.8 days with a geometric mean value of 329.0 day.

Table B.8.1.2.2-01 Laboratory half-lives and DegT90-values of bentazone at 10 °C

Soil	DegT50 original [days]	DegT90 original [days]	Temperature normalization factor - f_{temp} [-]	DegT50 normalized to 10 °C [days]	DegT90 normalized to 10 °C [days]	Source
Bruch West	33.0	109.6	2.58	85.1	282.8	II A 7.2.1/1 #2011/1000621
Li10	43.4	144.2	2.58	112.0	372.0	
LUFA 2.2	30.9	102.6	2.58	79.7	264.8	
LUFA 2.3	49.1	163.1	2.58	126.7	420.8	
Geometric mean	**38.4**	**127.5**		**99.0**	**329.0**	

B.8.1.2.1 Rate of degradation at 20ºC; laboratory studies (Annex IIA 7.2.1; Annex IIIA 9.1)

Second notifier data

STUDY IIA, 7.2.1/02

Characteristics

reference	:	Verhaar. H.J.M., 2012 (statement)	study type	:	Statement
year of execution	:	2012	incubation time	:	-
GLP statement	:	Yes (GMP)	nominal concentration	:	-
guideline	:	Not applicable	temperature	:	-
test substance	:	Not applicable	DT50	:	see results
purity	:	Not applicable		:	
soils	:	Not applicable	acceptability	:	**Not acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Study design

Soil degradation data for bentazone, determined under laboratory conditions, were reanalyzed

according to the FOCUS Degradation Kinetics Guidance Document (Sanco/ 10058/2005, v.2.0), as far as was possible based on the available information.

The 1996 Monograph summarizes a number of studies into the (route and) rate of degradation of bentazone in soil. None of the summaries present data that are required to perform a re-evaluation of the kinetic parameter derivation. Therefore, no recalculation was presented. A summary of the data from the DAR is given in table Table B.8.2.1-01 already. The author also provided a kinetic analyses of the data of Ebert, 2000. A graphical representation of the fit is given in Figure 1. This figure clearly shows that a SFO model adequately describes both the disappearance of bentazone and the appearance of N-methyl bentazone. The data obviously do not allow for the determination of a reliable degradation rate for N-methyl bentazone, but do provide a reliable estimate of the formation fraction. In the recalculation by the author , the decline of the parent compound and the appearance and disappearance of the metabolite was fitted (optimized) as a coupled system; more importantly, the author fit a decline curve for a laboratory soil degradation experiment with a fixed concentration at t=0, since he considers this concentration is known with a much higher accuracy than any of the later, analytical concentrations. The author is of the opinion that fitting an SFO decline curve with two free variables (i.e. C(0) and k) is not the correct approach. The DT50 derived for bentazone from these data was normalized to standard soil characteristics according to FOCUS Guidance (as incorporated in the DT50Convert _FOCUS Excel spreadsheet as prepared by M. Vanclooster). Numerical results are summarized in Table B.8.2.1-13. The visual fit of the observations is presented in the figure below.

figure B.8.1.2.1-02 bentazone and N-methylbentazone modelled against observed residue

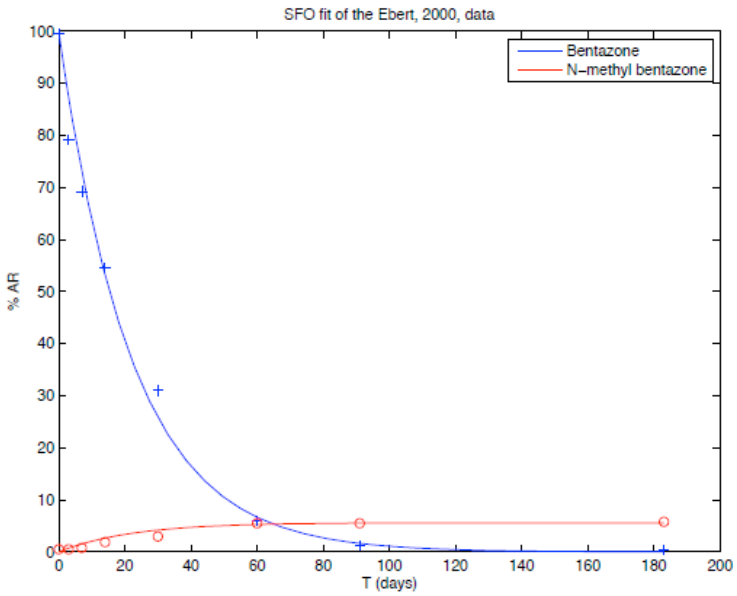
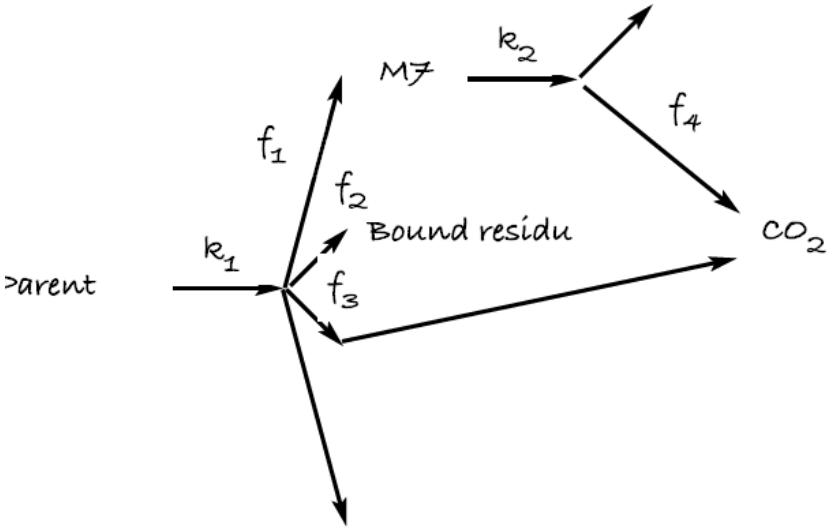


Table B.8.1.2.1-13 DT_{50} values for bentazone en N-methyl bentazone from study by Ebert (2000) calculated with the SFO model]

	Bentazone	N-methyl bentazone
DT50 (d)	15.4	-
Normalised DT50 (d)	14.2	-
F	-	0.057
X^2 (tab: 14.07)	6.89	1.78

Furthermore, the author of the statement re-evaluated study IIA, 7.1.1/06. These data were re-analysed using a custom MATLAB routine that allows for the simultaneous fitting of disappearance data of a parent compound and the formation and disappearance of metabolite(s). It gives the degradation half lives and formation fractions as fitted parameters, dependent on the specified degradation pathway. The pathway used to fit the presented data is the following:



A graphical representation of the fit is given in Figure B.8.2.1-02. Numerical results are summarized in Table B.8.2.1-14.

figure B.8.1.2.1-03 bentazone and N-methylbentazone modelled against observed residue

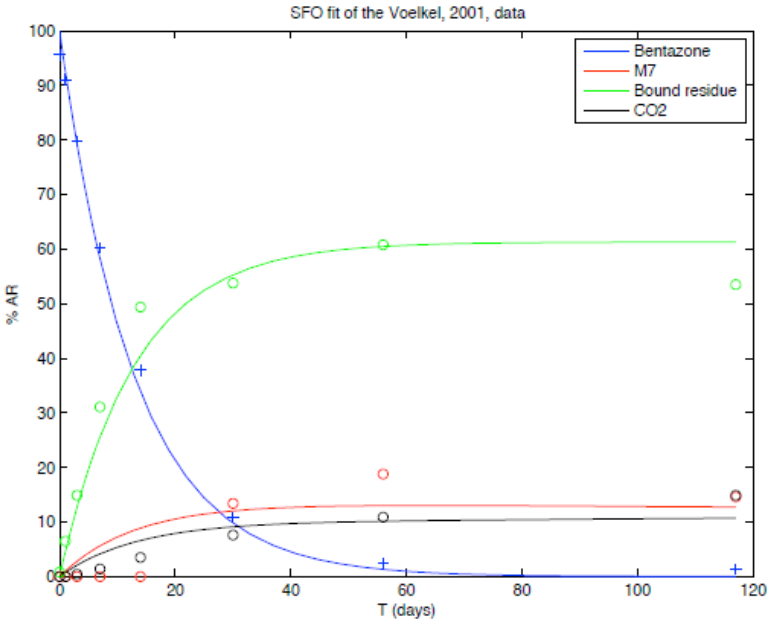


Table B.8.1.2.1-14 DT_{50} values for bentazone en M7 from study by Volkel (2001) calculated with the SFO model]

	Bentazone	M7	Bound residue	CO_2
DT50 (d)	8.95			-
Corrected DT50 (d)	17.3			-
f	-	0.135	0.61	0.1 (from parent
X^2 (tab: 14.07)	2.61	244	15.4	-

The data obviously do not allow for the determination of a reliable degradation rate for N-methyl bentazone, but do provide a reliable estimate of the formation fraction. In the recalculation by the author , the decline of the parent compound and the appearance and disappearance of the metabolite was fitted (optimized) as a coupled system; more importantly, the author fit a decline curve for a laboratory soil degradation experiment with a fixed concentration at t=0, since he considers this concentration is known with a much higher accuracy than any of the later, analytical concentrations. The author is of the opinion that fitting an SFO decline curve with two free variables (i.c. C(0) and k) is not the correct approach. The DT50 derived for bentazone from these data was normalized to standard soil characteristics according to FOCUS Guidance (as incorporated in the DT50Convert _FOCUS Excel spreadsheet as prepared by M. Vanclooster). Data and fit

results show that more than 70% of bentazone is converted into either CO_2 or bound residue. About 13.5% is converted into the metabolites that make up fraction M7; these metabolites appear to be persistent.

Comment

The statement provided by the applicant is not acceptable with regard to the derivation of the DT_{50} for the active substance bentazone. The applicant interpreted the FOCUS Kinetics guideline in a way thet is not in line with accepted risk assessment approaches. The values for metabolites and bound residue at t=0 should be added to the amount of parent substance at t=0. Furthermore, RMS does not agree with the statement: a decline curve for a laboratory soil degradation experiment is fitted with a fixed concentration at t=0, since this concentration is known with a much higher accuracy than any of the later, analytical concentrations. The author is of the opinion that fitting an SFO decline curve with two free variables (i.c. C(0) and k) is not the correct approach. RMS does not agree.

The calculations of the standardisation to normalised conditions are not provided in the statement. It is only referred to a spreadsheet that is not (made) available to RMS, therefore the correctness of the calculation cannot be judged. Last but not least no t-prob values are presented for the fitted parameters and therefore the statistical reliability of the fits is not proved.

B.8.1.2.2 Rate of degradation; Other studies

Main notifier data

STUDY IIA, 7.2.1/03

Characteristics

reference	:	Li K.-B. et al (2008b)	study type	:	Laboratory degradation
year of execution	:	2008	incubation time	:	30 days
GLP statement	:	no	nominal concentration	:	10 mg a.s./kg
guideline	:	none	temperature	:	25ºC
test substance	:	Atrazine; bentazone	DT50	:	-
purity	:	Bentazone >97%		:	
soils	:	Rhizosphere and non-rhizosphere	acceptability	:	**Not acceptable**
			Previous evaluation		Submitted for

				:	renewal (supplementary)

Study design

The rates of degradation of atrazine and bentazone separately as well as in combination were investigated in rhizosphere soil and non-rhizosphere soil originating from a corn field in China.

The experiments were performed at a temperature of 25 °C in the dark. Soil samples were sieved to 2 mm before use. Soils were treated with the herbicides at a concentration of 10 mg/kg soil (dry weight) which is considered to be 10 times the maximum recommended application rate used by the farmers.

The soil characteristics are summarized in Table B.8.1.2.1-15.

Table B.8.1.2.1-15 Properties of soil used to investigate degradation of atrazine and bentazone

Soil designation	**Huajiachi Campus**
Origin	Zhejiang University, Zhejiang Province, China
Particle size distribution [g/kg] sand silt clay textural class	 5.3 753 241.8 silty clay loam
Organic matter [g/kg]	36.4
pH	6.1

Duplicate samples were taken 2, 4, 6, 13, 21, 26, 36 and 47 days after treatment (DAT) from the rhizosphere and the non-rhizosphere soil. The autoclaved rhizosphere soil was sampled at 0.7, 3, 4, 6, 9, 12, 15, 17, 20, 26, and 30 DAT. Collected samples were immediately frozen and stored at -20 °C until analysis.

For analysis the soil was extracted three times with a methanol / water mixture (9:1, v/v). The combined extracts were concentrated by rotary evaporation at 45°C to the aqueous phase. The aqueous extracts were three times partitioned against dichloromethane. The combined dichloromethane phase was concentrated to dryness, re-dissolved in a methanol / water mixture (66:44) and analysed by HPLC.

Results

No degradation of either atrazine or bentazone was observed in autoclaved rhizosphere soil, whereas in all experiments using non-autoclaved soil a continuous decrease of the herbicides was found. This indicates degradation of both substances by biotic processes.The resulting degradation curves were fitted to the first-order model $\ln(c_t/c_0) = -k\ (t - t_{lag})$.

When comparing rhizosphere and non-rhizosphere soil for separately applied herbicides, a faster degradation was found for rhizosphere soil in case of atrazine but also a small lag-phase, which was not observed in non-rhizosphere soil. For bentazone, the degradation rate for rhizosphere and non-rhizosphere soil was comparable; although a lag-phase (6.8 days) was found in non-rhizosphere soil.

Application of a combination of both herbicides led to a diminished degradation rate for bentazone and an elongated lag-phase for both herbicides. This effect was lessened by addition of Tween 20 to the combination (see table Table B.8.1.2.1-16).

Table B.8.1.2.1-16 Degradation parameters of atrazine and bentazone

Herbicide	**Treatment**	**K [d^{-1}]**	**$t_{1/2}$ [d]**	**t_{lag} [d]**	**r^2**
Atrazine	Rhizosphere soil	0.02694 (0.00047)[1)]a [2)]	25.7	2.0	0.99
	Non-rhizosphere soil	0.02386 (0.00213) b	29.1	0.0	0.97
	Rhizosphere soil plus bentazone	0.02249 (0.00171) c	30.8	6.7	0.99
	Rhizosphere soil plus bentazone + Tween 20	0.02255 (0.00339) c	30.7	3.2	0.95
Bentazone	Rhizosphere soil	0.03432 (0.00272) a	20.2	0.0	0.98
	Non-rhizosphere soil	0.03475 (0.00568) a	19.9	6.8	0.95
	Rhizosphere soil plus atrazine	0.02744 (0.00368) b	25.2	6.6	0.97

Herbicide	Treatment	K [d^{-1}]	$t_{1/2}$ [d]	t_{lag} [d]	r^2
	Rhizosphere soil plus atrazine + Tween 20	0.03233 (0.00430) a	21.4	4.2	0.96

Degradation parameters are derived from the model $\ln(c_t / c_0) = k(t - t_{lag})$ and $t_{1/2} = \ln 2/k$

[1)] Values in parentheses are standard deviation.

[2)] Means for a given herbicide followed by the same letter are not significantly different (P = 0.05) using t-test.

Although the authors postulated a significant difference in degradation rates of bentazone when applied separately or in combination with atrazine, the calculated degradation rates overall were very similar. Irrespective of the kind of incubation, the half-lives of bentazone ranged between 20 and 25 days.

Comment

This is a non guideline non GLP study on typical degradation of bentazone in the rhizosphere. The study is not considered acceptable for risk assessment.

STUDY IIA, 7.2.1/04

Characteristics

reference	:	Rodriguez Cruz M.S. et al (2008b)	study type	:	Public literature
year of execution	:	2008	incubation time	:	127 days
GLP statement	:	no	nominal concentration	:	5 mg a.s./kg
guideline	:	none	temperature	:	15ºC
test substance	:	bentazone	DT50	:	-
purity	:	Basagran (87% w/w)		:	
soils	:	5 depths at 40% MWHC	acceptability	:	**Additional**
			Previous evaluation	:	Submitted for renewal (supplementary)

Study design

To study the spatial variability of bentazone degradation with soil depth, two experiments were conducted using soil from a farm in, Warwickshire, UK.

For investigation of vertical variability in degradation, soil from five depths (0 - 10, 20 - 30, 40 - 50, 60 - 70, 70 - 80 cm) and three sampling points within the trial field was sampled, sieved to <3mm and treated with Basagran (containing 87% bentazone) to reach a concentration of 5 mg a.s. / kg soil. Samples were adjusted to 40% MWHC (ca.15.5% moisture content) and incubated at 15°C in the dark.

Soil properties are reported in table B.8.1.2.1-17

Table B.8.1.2.1-17 Change in key soil properties and bentazone degradation with soil depth

Soil depth (cm)	**OM (%)**	**pH[a]**	**Biomass [mg C kg^{-1} soil][a]**	**Dehydrogenase [µg TPF g^{-1} soil][a]**	**DT_{50} [d][b]**
0 - 10	2.62	7.06	65.5	43.1	56
20 - 30	2.33	7.13	64.5	22.9	65
40 - 50	1.98	7.79	36.4	10.7	178
60 - 70	1.66	7.98	21.5	7.48	306
70 - 80	1.35	8.09	19.1	6.60	515
Least significant difference (p>0.05)	0.29	0.45	16.0	14.7	178
Significance of effect of depth	***	**	***	***	***

Data represents mean of three sampling locations at each depth.

TPF Triphenyl formazan.

NS, not significant.

**Significant, p<0.01.

***Significant, p< 0.001

[a] From Bending and Rodríguez-Cruz (2007).

[b] obtained from sieved soil.

Samples were taken in regular intervals within a period of three months and analysed for bentazone. Organic matter content, pH, biomass, and dehydrogenase activity and K_d were determined for all soil samples.

For investigation of vertical and horizontal variability in degradation of bentazone, soil was sampled at two depths (0 - 10 and 50 - 60 cm) at ten different sampling points within the trial plot. Samples were taken as disturbed and undisturbed soil cores. The soil from disturbed soil cores was sieved to <3mm, adjusted to 40% MWHC (ca. 15.5% moisture content) and treated with Basagran as described above. Undisturbed soil cores were treated at the same nominal concentration by injections into the soil core and incubated vertically. Moisture content in the undisturbed soil cores was not standardized across the cores but was found to be 13.3% and 16.7% at harvest in cores from 0 - 10 and 50 - 60 cm, respectively.

All samples prepared in this second experiment were analysed for bentazone after 127 d of incubation at 15°C in the dark.

Results

In the first experiment bentazone decreased with time in all soil depths investigated. As bentazone has a low vapor pressure and a very low sorption in soil, it was concluded that the dissipation of the substance reflects degradation. The degradation of bentazone was found to follow first order kinetics in the top soils (0 - 10, 20 - 30 cm). In the subsoils (30 - 40, 50 - 60, 70 - 80 cm) the degradation was fitted to a linear model. DT_{50} values ranged from 56 to 515 days and were found to increase with soil depth. Furthermore DT_{50} values were found to correlate with organic matter content, pH, biomass, dehydrogenase activity and K_d.

In the second experiment, a faster degradation was found in the intact soil cores for the 0 - 10 cm depth compared to the sieved soil, reflected in the lower amount of bentazone remaining in the soil of intact cores (6.63%) compared to sieved soil (17.2%). In the subsoil (50 - 60 cm depth), no significant difference was found between the intact cores and sieved soil.

Comment

Agree with applicant that the experiments described in this publication do not follow common guidelines used for pesticide registration and were not performed according to GLP rules, they are considered informative for degradation in deeper soil layers.

STUDY IIA, 7.2.1/05

Characteristics

reference	:	Smelt J.H. (2003b)	study type	:	Fiel-,subsoil degradation
year of execution	:	1999-2002	incubation time	:	462 days
GLP statement	:	yes	nominal concentration	:	1 mg a.s./kg, 0.01 or 0.02 mg/kg for subsoil
guideline	:	none	temperature	:	15ºC, 10ºC for subsoil
test substance	:	bentazone	DT50	:	-
purity	:	Not reported		:	
soils	:	5 field soils 3 depths	acceptability	:	**Additional**
			Previous evaluation	:	Submitted for renewal (supplementary)

Study design

The dissipation of BAS 351 H - bentazone in soil systems was investigated in freshly collected Dutch soils from three depths (topsoil, unsaturated subsoil, saturated subsoil) of fields in five relevant agricultural regions. The fields were carefully selected considering soil properties, planted crops and bentazone use in the respective areas. Also hydrogeological information was included. From each field, soil samples from three soil depths (top layer, unsaturated subsoil layer, water-saturated subsoil) were collected. Soil from the top layer (0 - 25 cm) and the unsaturated subsoil layer (40 - 90 cm) were sampled with an auger, while the water-saturated subsoil (2.2 - 4.4 m depth depending on groundwater table) was sampled using a special sampling equipment and care was taken to ensure no contamination with surface soil. Saturated subsoils of those sites which showed more anaerobic conditions were transported and stored under Argon gas atmosphere.

The site characteristics of the sampled soils are presented in B.8.1.2.1-18.

Table B.8.1.2.1-18 Soil characteristics of the sampled soils

Location	**Vredepeel**	**Vlagtwedde**	**Buurse**
Soil class (Dutch	podzol	podzol	dikke enkeerdgrond

specification)									
Depth (cm)	0-25	50-80	220-310 *	0-25	50-80	235-320 *	0-25	50-80	310-400
Clay (< 2 µm) %	2.7	1.9	2.1	2.4	2.4	2.6	2.1	3.2	2.3
Silt (2-50 µm) %	7.8	0.1	0.6	10.4	2.9	1.2	5.8	7.0	0.0
Sand (50-2000 µm) %	84.7	97.5	97.2	81.3	94.4	96.1	88.4	84.1	97.6
Organic Carbon (g/100 g dry soil)	2.89	0.31	0.04	5.52	0.15	0.04	2.43	3.73	0.03
$CaCO_3$ (g/100g dry soil)	0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
pH(H_2O)	6.4	5.3	5.5	5.6	5.3	5.6	5.8	4.8	5.2
pH(KCl)	6.2	4.5	4.5	4.9	4.4	4.4	5.0	4.0	4.6
CEC ($cmol^+$/kg dry soil)	8.9	0.3	< 0.1	8.6	< 0.1	< 0.1	4.9	4.1	< 0.1
Microbial biomass (mg C/100 g dry soil)	15.6	-	-	18.1	-	-	13.6	2.3	-
Saturated subsoil data [§]									
Temperature (°C)	7.9			7.4 - 8.1			8.0		
pH	4.9 – 5.07			5.06 - 5.5			4.37 – 4.45		
Oxygen (mg/L)	0.48 – 0.91			0.40 - 0.71			8.9 – 9.6		
Redox potential (mV) **	263 - 344			231 - 251			437 - 515		

Location	**Esbeek**			**Roosendaal**		
Soil class (Dutch specification)	dikke enkeerdgrond			podzol		
Depth (cm)	0-25	40-70	320-420	0-25	60-90	320-410 *
Clay (< 2 µm) %	3.1	2.8	2.3	3.1	4.0	3.7

Silt (2-50 µm) %	15.4	15.6	0.8	6.2	1.2	0.3
Sand (50-2000 µm) %	78.1	79.9	96.9	86.8	94.1	95.8
Organic Carbon (g/100 g dry soil)	2.15	1.09	0.02	2.24	0.24	0.04
$CaCO_3$ (g/100g dry soil)	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1
pH(H_2O)	5.8	5.5	5.5	6.1	6.2	5.0
pH(KCl)	4.8	4.6	4.5	5.4	5.1	4.3
CEC ($cmol^+$/kg dry soil)	2.6	0.8	< 0.1	5.3	0.4	0.7
Microbial biomass (mg C/100 g dry soil)	20.8	1.8	-	25.8	-	-
Saturated subsoil data [§]						
Temperature (°C)	9.8 – 9.9			9.2 – 10.2		
pH	4.57 – 5.06			4.19 – 4.45		
Oxygen (mg/L)	6.0 – 7.1			0.53 – 0.76		
Redox potential (mV) **	408 - 492			270 – 323		

[§] range of 2-3 measurements at the date of collection, *in situ* measurements

\- not measured

* anaerobic conditions in saturated soil layer

** corrected to standard hydrogen electrode

Subsamples of approximately 100 g (dry weight) of the topsoil layers and non-saturated subsoil layers were incubated in the dark at a temperature of 15 °C, and of the saturated layers at 10 °C. After a pre-incubation time of 5 - 7 days, bentazone was applied, giving an initial content of 1 mg/kg dry soil in topsoil and 0.01 or 0.02 mg/kg in the unsaturated subsoils.

For the saturated subsoils, subsamples of average 170-200 g (dry weight) were incubated under saturated conditions with 48-59 mL groundwater at a temperature of 10 °C in closed serum bottles with either a nitrogen atmosphere in the headspace (for three anaerobic subsoils) or in not airtight closed bottles with atmospheric condition (for two aerobic subsoils). After a pre-incubation time of 5 - 7 days, bentazone was

applied, giving an initial content of 0.005 mg/kg dry soil. Redox potential and pH were monitored over the study duration. No clear changes were measured in either the aerobic saturated subsoils or the anaerobic saturated subsoils.

Duplicate samples were analysed at increasing time intervals, for a maximum duration of 462 days. Sampling were adapted to the expected or observed rates of decrease of bentazone. Topsoils and saturated subsoils were extracted with methanol, unsaturated subsoils were extracted with 0.01 M $CaCl_2$. For the saturated subsoils, after extraction a concentration step was included. For the unsaturated subsoils, after extraction, the slurry was centrifuged, the extract acidified and then extracted with CH_2Cl_2. the dried residue was also analysed for bentazone. The method was validated within the study as follows: during the incubation experiment, in each analytical series at least one soil type was spiked with bentazone at the level of application at the start of the incubations (100 µg for the subsoils and 1.0 µg for the two subsoils) and a lower level near the limit of quantification (2 µg for the topsoils and 0.1 µg for the subsoils).

Results

Results from the recovery tests for the high doses indicated that recoveries were high (79.8 – 105.8 % of applied), except for the unsaturated subsoils extracted with 0.01 M $CaCl_2$.

Recoveries for the low doses were between 69.1 and 165.6 %. A relatively high standard deviation in the unsaturated subsoil with MeOH extraction and the water-saturated subsoils was observed and is mainly caused by one sample in each series. However, the blank sample showed also a peak at the retention window of bentazone at that time. Corrections for this would deliver reasonable extraction efficiencies.

The results of bentazone analyses showed that degradation of bentazone (at 15°C) could be observed in the topsoils as well as in the unsaturated subsoils of all five locations. The DT_{50} in topsoils was approximately two weeks for four locations, and four weeks for the fifth location. The DT_{50} in unsaturated subsoils was between approximately one week and eight weeks for four locations, and 32 weeks for the fifth location.

In saturated subsoils (incubated at 10°C), bentazone degradation was slowed down considerably, as the remaining fraction of bentazone was still between 54.9 % and 92.9 % at the end of the study.

Monitoring of redox potential and pH over the study duration indicated that conditions were within the ranges measured in the field, and no clear changes were measured with incubation time in either the aerobic subsoils or the anaerobic subsoils.

Comments

The study provides additional information about the degradation of bentazone in aerobic and anaerobic subsoils. The study is not a standard degradation study under adopted guidelines and is considered additional information.

STUDY IIA, 7.2.1/06

Characteristics

reference	:	Van de Veen J.R. (2003b)	study type	:	Kinetic evaluation
year of execution	:	2003	incubation time	:	See 7.2.1/05
GLP statement	:	n.r.	nominal concentration	:	See 7.2.1/05
guideline	:	none	temperature	:	See 7.2.1/05
test substance	:	-	DT50	:	-
purity	:	-		:	
soils	:	See 7.2.1/05	acceptability	:	**Additional**
			Previous evaluation	:	Submitted for renewal (supplementary)

Study design

Since the degradation rates mentioned in the above summarized study (*Smelt*, 2003) were only rough graphical estimations from the degradation curves, an extra kinetic evaluation was performed in this study.

The dissipation of bentazone in different soil layers was evaluated by applying a 1st order kinetic model to the data generated in Smelt (2003). ModelMaker 3.0.4 was used to implement a single first-order (SFO) compartmental model, and the resulting half-lives were converted to half-lives at a reference temperature of 20 °C, using a Q_{10} value of 2.2. The 1st order rate constant (k_{12}) was estimated using the non-linear regression algorithm of Marquardt-Levenberg with option Least Squares as implemented in the ModelMaker 3.0.4 package. Initial M_0 and k were set to 100 and 0.01, respectively. A t-test was employed ($\alpha = 0.05$) to identify the probability that a parameter was not significantly different from zero.

Results

The resulting degradation parameters and DT_{50} values confirmed rapid degradation of bentazone in the topsoil with half-lives (at reference temperature 20 °C) of 8.8-21.6 days, and in the unsaturated subsoil with half-lives of 6.4 - 48.2 days (and one value of 196 days, where degradation was slow but with a continuous decrease). However, in the water-saturated subsoil the calculated degradation rates were much longer showing values between 270 and 1380 days

The resulting parameter values, corresponding DT_{50} values and r^2 of the parameter estimation as well as the results of normalisation are summarized in Table B.8.1.2.1-19.

Table B.8.1.2.1-19 Half-lives of bentazone in Dutch soil profiles

Location	Depth	k (d^{-1})	r^2	Half-life at experimental temp. condition [d]	Half-life at reference temp. 20°C [d]
Vredepeel	topsoil	0.021655	0.998	32.0 at 15°C	**21.58 20.1**
	50-80 cm	0.018395	0.978	37.7 at 15°C	**25.40**
	water saturated *	0.000115	0.993	3036.1 at 10°C	**1380.1**
Vlagtwedde	topsoil	0.053368	0.998	13.0 at 15°C	**8.76 8.2**
	50-80 cm	0.0023843	0.990	290.7 at 15°C	**196.00**
	water saturated *	0.0011033	0.965	628.2 at 10°C	**285.6**
Buurse	topsoil	0.037206	0.985	18.6 at 15°C	**12.56 11.7**
	50-80 cm	0.073049	0.947	9.5 at 15°C	**6.40**
	water saturated *	0.000312	0.983	595.0 at 10°C	**270.4**
Esbeek	topsoil	0.033156	0.993	20.9 at 15°C	**14.09 13.1**
	40-70 cm	0.031523	0.994	22.0 at 15°C	**14.82**
	water saturated *	0.000311	0.982	1288.4 at 10°C	**585.7**
Roosendaal	topsoil	0.040992	0.995	16.9 at 25°C	**11.40 10.6**
	60-90 cm	0.0096888	0.995	71.5 at 15°C	**48.23**
	water saturated *	0.00052295	0.984	1325.5 at 10°C	**602.5**

*incubated at 10 °C

The dissipation of bentazone in different soil layers was well described by the simple 1st order kinetic model. The high coefficient of determination (r^2= 0.95-0.99), low type-I error rate (<0.001) and good agreement between measured and calculated dissipation curves gave evidence of a good overall fit of the 1st order kinetic model.

The resulting degradation parameters and DT_{50} values confirmed rapid dissipation of bentazone with half-lives (at reference temperature 20 °C) in the soil phase of the topsoil of 8.8-21.6 days, in the unsaturated

subsoil of 6.4 - 48.2 days (and one value of 196 days, where degradation was slow but with a continuous decrease), and in the water-saturated subsoil of 270 - 1380 days.

Comments

Apparently a t-test was employed ($\alpha = 0.05$) to identify the probability that a parameter was not significantly different from zero. The results thereof were not reported.

The study is pre FOCUS Kinetics The normalisation is not according to currently accepted approaches. The study is considered additional.

STUDY IIA, 7.2.1/07

Characteristics

reference	:	Leistra (2001)	study type	:	Soil transformation
year of execution	:	2001	incubation time	:	278 days
GLP statement	:	No (publication)	nominal concentration	:	0.01-1.32 µg/g
guideline	:	none	temperature	:	15 ºC
test substance	:	bentazone	DT50	:	See results
purity	:	99.2%		:	
soils	:	Humic sandy soil	acceptability	:	**Additional**
			Previous evaluation	:	Submitted for renewal (supplementary)

Study design

The transformation of bentazone was followed in samples from four layers and three collection sites of a humic sandy soil from an experimental field near Vredepeel (Netherlands). It was expected that the depth of the water table would fluctuate substantially in this period, from comparatively deep in late summer to shallower in early spring. The depth to the groundwater table was measured via two groundwater tubes, with pressure gauge and data logger. During the collection period, water-table depth range between 0.8 and 1.4m.

The investigated soil layers included the humic sandy plough layer (0-0.25m, layer A, two dates), the sandy vadose zone with fluctuating water table (0.5-0.75m, layer B, four dates and 1.0-1.2 m, Iayer C, four dates)

and the permanently water-saturated sandy subsoil (2.0-2.5m, layer D, one date). Soil samples were collected as indicated at up to four dates within a seven-month period and transferred to the laboratory.

Incubation samples for the layers A to C were generated by mixing equal quantities of soil from the three collection sites X, Y and Z. For layer D samples from the different sites were incubated separately.

For each incubation, 39 g were weighed in for layers A, B and C and 98 g for layer D and treated with bentazone at contents between 0.0109 and 1.32 µg/g dry soil. The soil was incubated at the constant temperature of 15°C in the dark at similar moisture conditions as found in the field at sampling. Aliquots of the water-saturated subsoil were incubated under anaerobic conditions. Furthermore, to check biological influence, selected soil samples were gamma-sterilized and treated with bentazone as well.

Soil characteristics are shown in Table B.8.1.2.1-20.

Table B.8.1.2.1-20 Properties of soil layers used to investigate degradation of bentazone

Layer	**A (0-0.25m)**	**B (0.50-0.75 m)**	**C (1.0-1.2 m)**	**D (2.0-2.5 m)**
Origin	Vredepeel, The Netherlands	Vredepeel, The Netherlands	Vredepeel, The Netherlands	Vredepeel, The Netherlands
Particle size distribution [%]				
	3.1	0.4	0.4	8.9
silt 0.002 – 0.050 mm	3.6	2.3	2.2	3.6
clay < 0.002 mm	sand	sand	sand	sand
textural class				
Organic matter [%]	4.8	0.4	0.2	0.4
$CaCO_3$ [%]	0.1	<0.1	<0.1	<0.1
pH [KCl]	5.3	4.5	4.6	4.5
pH [H_2O]	6.5	5.9	5.9	5.1
In soil horizon	A_p	B_e/C_e	C_e	C_f

The redox potential in the four layers was measured in the field on 12 days. The redox potential in layers A, B and C was about 500 mV, indicating that these layers were aerobic. In the permanently water-saturated layer D, the redox potential was about 200 mV, which indicates moderately anaerobic conditions.

Aqueous solutions of bentazone were prepared at different concentrations, and 1 mL of solution was applied to the soil in each tube. Using a syringe, the solution was distributed in small drops over the enlarged soil surface in the tilted tube, after which the contents of the tube were mixed by shaking and rolling. The dose was checked by adding 1 mL of the aqueous solution to dichloromethane after each tenth addition to the soil.

Samples were incubated for periods between 84 and 278 days in the dark at 15°C. In some of the series, bentazone was also incubated in gamma-irradiated soil materials. For this purpose, the soil was weighed into serum bottles provided with a rubber septum and a screw cap. The soils were exposed to gamma irradiation. At distinct sampling times two soil batches of the incubation series for layers A to C were extracted with water and the further processed extract analysed for bentazone by HPLC. Single batches were extracted for the layer D series for each of the collection sites X, Y and Z.. Additionally, the physico-chemical characteristics of the soil (texture, pH, $CaCO_3$, organic carbon, dissolved organic carbon, nitrogen and water extractable phosphorous) as well as the microbial biomass were determined in subsamples of the collected soil.

Results

The first-order transformation rates determined varied between the different soil layers, with the date of soil collection and with the initial content of bentazone applied.

The highest transformation rates were found in layer A, followed by the water-saturated layer D and then by layers B and C. In general, the lower the applied concentration of the test item the higher were the transformation rates found. For the sterilized soil, transformation was remarkably slowed down (Layer A and D) or not measurable (Layers B and C) suggesting that transformation is mainly a microbial process.

A compilation of degradation parameters (degradation rates and half-lives) of all investigated soil samples is given in Table B.8.1.2.1-21.

For ease of comparison the degradations of all trials were described as first-order kinetics although some of the A layer degradation curves were rather following kinetics with changing degradation constants.

Generally, soil sterilization was remarkably slowing down degradation of bentazone suggesting that the observed bentazone dissipation is basically a microbial process. As a general tendency it was found that the degradation rates increased with lower initial bentazone content. The authors discuss as possible interpretations that higher levels of bentazone inhibit microbial activity or a comparatively low density of micro-organisms was able to transform bentazone. Transformation rates showed the same trend as some of the soil factors i.e. the organic carbon content and the water soluble phosphorous content, although a clear relation was difficult to see.

Table B.8.1.2.1-21 First-order rate constants and half-lives for the transformation of bentazone in soil material from the four layers of the Vredepeel field. Initial contents on the basis of dry soil

Layer	Month of collection	Fresh/ sterilized	Initial content [µg/g]	Rate constant [$days^{-1}$]	Standard error [$days^{-1}$]	Half-life [days]

Layer	Month of collection	Fresh/ sterilized	Initial content [µg/g]	Rate constant [days^{-1}]	Standard error [days^{-1}]	Half-life [days]
0 - 25 cm	November 1996	Fresh	1.32	0.0299	0.0007	23.2
	April 1997	Fresh	0.117	0.0694	00065	10.0
	April 1997	Fresh	1.16	0.0437	0.0021	15.8
	November 1996	Sterilized	1.32	0.0044	0.0008	156
50 - 75 cm	September 1996	Fresh	0.0116	0.0182	0.0012	38.0
	November 1996	Fresh	0.0119	0.0047	0.0004	147
	February 1997	Fresh	0.0109	0 0067	0.0002	104
	April 1997	Fresh	0.0130	0.0074	0.0007	93.4
	April 1997	Fresh	0.110	0.0030	0.0002	230
	November 1996	Sterilized	0.0119	nd[a]		
1.0 - 1.2 m	September 1996	Fresh	0.0126	0.00279	0.00021	248
	November 1996	Fresh	0.0126	0.00372	0.00040	186
	February 1997	Fresh	0.0114	0.00340	0.00022	204
	April 1997	Fresh	0.0150	0.00224	0.00041	309
	April1997	Fresh	0.127	0.00199	0.00027	349
	November 1996	Sterilized	0.0126	nd[a]		
2.0 - 2.5 m site X	April 1997	Fresh	0.0269	0.0236	0.0025	29.3
			0.0515	0.0195	0.0033	35.6
site Y	April 1997	Fresh	0.0269	0.0191	0.0040	36.4
			0.0515	0.0080	0.0011	86.9
site Z	April 1997	Fresh	0.0269	0.0240	0.0039	28.9
			0.0515	0.0159	0.0030	43.5
site X	April 1997	Sterilized	0.0354	0.00219	0.00117	317
site Y	April 1997	Sterilized	0.0354	0.00268	0.00104	259

Layer	Month of collection	Fresh/ sterilized	Initial content [µg/g]	Rate constant [days^{-1}]	Standard error [days^{-1}]	Half-life [days]
site Z	April 1997	Sterilized	0.0354	0.00853	0.00308	81.3

Comment

Overall, the results of the present publication show that the degradation of bentazone in soil decreases with increasing soil depths. Major factor for the degradation rates proved to be microbial activity which depends on various factors like temperature and nutrient availability, and if suitable conditions exist allowing for degradation processes even in deeper subsoils, especially at lower bentazone concentrations which can be assumed at soil depths.

B.8.1.2.3 Rate of degradation at 20ºC; laboratory studies (Annex IIA 7.2.3; Annex IIIA 9.1)

Main notifier data

metabolite

STUDY IIA, 7.2.3/01

Characteristics

reference	:	Class T., 2005b	study type	:	aerobic degradation
year of execution	:	2004	incubation time	:	up to 181 d
GLP statement	:	Yes	nominal concentration	:	0.64 mg/kg
guideline	:	Incl OECD 307 (2002)	temperature	:	20±2°C
test substance	:	[N-methyl bentazone.	DT50	:	see results
purity	:	chemical purity 99.8, (batch no. 2235-09)		:	
soils	:	loam, sand, loam and clay loam	acceptability	:	**acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Study design

The degradation rate of N-methyl-bentazone under aerobic conditions was investigated in three different soils at a temperature of 20°C. The soils were freshly collected from the field. Soil properties are reported in table B.8.2.1-02.

The soils were treated with a nominal rate of 0.64 mg non labelled N-methyl-bentazone per kg dry soil which was calculated assuming a bentazone application rate of 1.5 kg/ha and a resulting metabolite formation of max 0.32 kg/ha. Incubations were in the dark at soil moisture of 40-50 % of the maximum water holding capacity. Samples were taken at 0, 1, 2, 3, 4, 6, 8, 15, 30, 37, 60, 90, 120, 150 and 181 days after treatment (DAT).

Table B.8.1.2.3-01 Properties of soils used to investigate the degradation rate of N-methyl-bentazone under aerobic conditions

Soil designation	**LUFA Speyer 2.2 (F221904)**	**LUFA Speyer 3A (F3A 1804)**	**PTRL soil**
Origin	Hanhofen, Rhineland-Palatinate Germany,	Altlußheim; Baden-Württemberg Germany	Ulm-Ermingen, Baden-Württemberg, Germany
USDA Particle size distribution [%]			
sand 0.050 – 2 mm	77.5	48.5	30.1
silt 0.002 – 0.050 mm	14.6	34.6	42.2
clay < 0.002 mm	7.9	16.9	27.7
textural class	loamy sand	loam	clay loam
Organic C [%]	2.29	2.2	1.31
Organic matter [%] *	3.95	3.79	2.26
pH [$CaCl_2$]	5.7	7.1	6.8
Cation exchange capacity [$cmol^+$ / kg]	11	17	15
Maximum water holding capacity [g/100g dry soil]	48.4	51.2	45.5
Microbial biomass (6 days before start of study) [mg C/100g dry soil]	19	30	28
Microbial biomass (after 62 days) [mg C/100g dry soil]	56	94	53

Microbial biomass (after 120 days) [mg C/100g dry soil]	46	89	39

The soil samples were extracted with 100 mL of methanol by shaking for 0.5 h. Samples for analysis by the ion trap LCQ LC/MS/MS were centrifuged. A 20 mL aliquot of the raw extract was mixed with 20 mL of water and concentrated on a SPE cartridge. The retained analyte was eluted from the SPE material with 5 mL of acetone. The acetone eluate was further eluted through a SPE cartridge. The eluate was concentrated to dryness and the residue dissolved in methanol/water (1/1 v/v) for LC/MS/MS analysis. Samples intended for analysis by the API 3000 LC/MS/MS were prepared without SPE clean-up by diluting the final extract with methanol and water (1/4/5, v/v/v).

Two different kinetic models were applied to describe the degradation of N-methyl-bentazone in soil: single first-order kinetics (SFO) and first-order multi-compartment kinetics (FOMC, also known as Gustafson-Holden model). The Solver function in Microsoft Excel was used to optimise free parameters, i.e. the initial applied amount M_0 and the degradation rate constant k (SFO model) or the kinetic parameters α and β (FOMC model), respectively.

Results

The soil analysis method was validated before start of the soil experiment at the limit of quantification (LOQ) of 0.05 mg/kg and at the initial application rate of 0.64 mg/kg, resulting in average recoveries of 102 % (±13 %, n=9) and 98 % (±11 %, n=9), respectively. The average recovery over the entire 181-days incubation period determined by processing freshly fortified soil (0.64 mg/kg) was 103 % (±11 %, n=21, using the LCQ of the LC/MS/MS instrument) and 112 % (±8 %, n=21, using an LC/MS/MS analysis, without any clean-up of the raw extracts).

The results of HPLC-MS analysis are shown in Table B.8.1.2.3-02.

Table B.8.1.2.3-02 Degradation of N-methyl-bentazone in soil incubated under aerobic condition at 20°C

Days after treatment	**LUFA Speyer 2.2 (F221904)**		**LUFA Speyer 3A (F3A 1804)**		**PTRL soil**	
	N-methyl-bentazone [mg/kg]	**Recovery [%]**	**N-methyl-bentazone [mg/kg]**	**Recovery [%]**	**N-methyl-bentazone [mg/kg]**	**Recovery [%]**
0 repl. 1	**0.705***	110%	**0.690***	108%	**0.702***	110%
0 repl. 2	**0.727***	114%	**0.692***	108%	**0.719***	112%

1	**0.702**	110%	**0.675**	106%	**0.713**	111%
2	**0.693**	108%	**0.660**	103%	**n.p.**	n.p.
3	**0.656**	102%	**0.640**	100%	**0.693**	108%
4	**0.680**	106%	**0.636**	99%	**0.642**	100%
6	**0.702**	110%	**0.594**	93%	**0.658**	103%
8	**0.675**	106%	**0.570**	89%	**0.616**	96%
15	**0.638**	100%	**0.455**	71%	**0.563**	88%
30	**0.620**	97%	**0.444**	69%	**0.458**	72%
37	**0.572**	89%	**0.442**	69%	**0.433**	68%
60	**0.563**	88%	*0.389*	61%	**0.385**	60%
60	**0.576**	90%	*0.405*	63%	**0.367**	57%
90	**0.451**	70%	**0.068**	11%	*0.052*	8%
120 repl. 1	**0.451**	70%	*0.363*	57%	**0.172**	27%
120 repl. 2	**0.449**	70%	*0.345*	54%	*0.330*	52%
150 repl. 1	**0.323**	51%	**0.020**	3.1%	**0.008**	1%
150 repl. 2	**0.350**	55%	**0.018**	2.8%	**0.009**	1%
181 repl. 1	**0.275**	43%	**0.016**	2.5%	**0.010**	2%
181 repl. 2	**0.297**	46%	**0.017**	2.6%	**0.007**	1%

n.p. not performed

Values in *italics* were defined as outliers for kinetic analysis

R_{dosed} = 0.64 mg/kg

* mean of two values

The goodness-of-fit for the optimised degradation curves was assessed by visual and statistical methods. The minimum error level to pass the χ^2 test was defined as the criterion for the best-fit model, i.e. the model with the lowest error level was selected.

The estimated best-fit DT_{50} and DT_{90} values for N-methyl-bentazone in aerobic soils are shown in Table B.8.2.1-03.

Table B.8.1.2.3-03 Estimated DT_{50} and DT_{90} and χ^2 - error levels

Soil	SFO kinetics results			SFO statistical indices		FOMC statistical indices	
	k [d^{-1}]	DT_{50} [d]	DT_{90} [d]	r^2	χ^2 error [%]	r^2	χ^2 error [%]
LUFA Speyer 2.2 (Loamy Sand)	0.0045	153	508	0.9727	3.2	0.9727	3.3
LUFA Speyer 3A (Loam)	0.0183	38	126	0.9815	7.9	0.9815	8.2
PTRL Soil (Clay Loam)	0.0141	49	164	0.9756	6.9	0.9756	7.2

Fig B.8.1.2.3-01 Degradation of N-methyl-bentazone in soil incubated under aerobic condition at 20°C, LUFA Speyer soil (3A and 2.2).

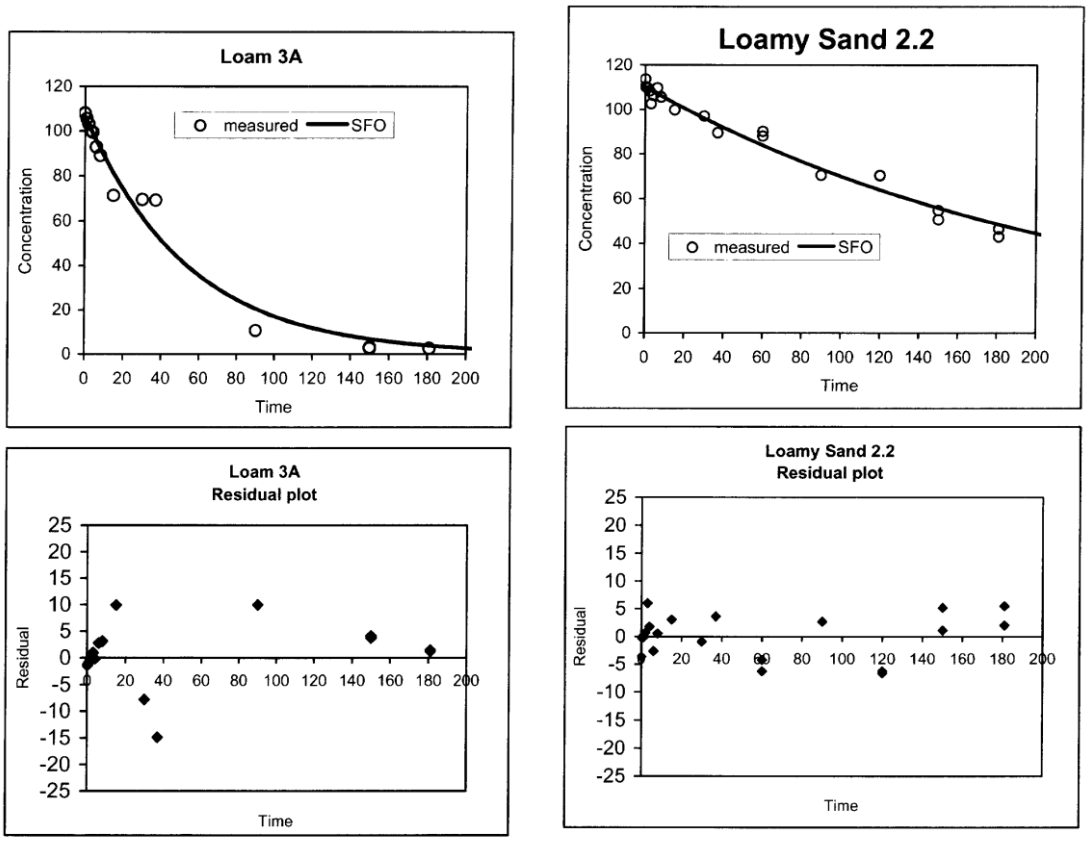
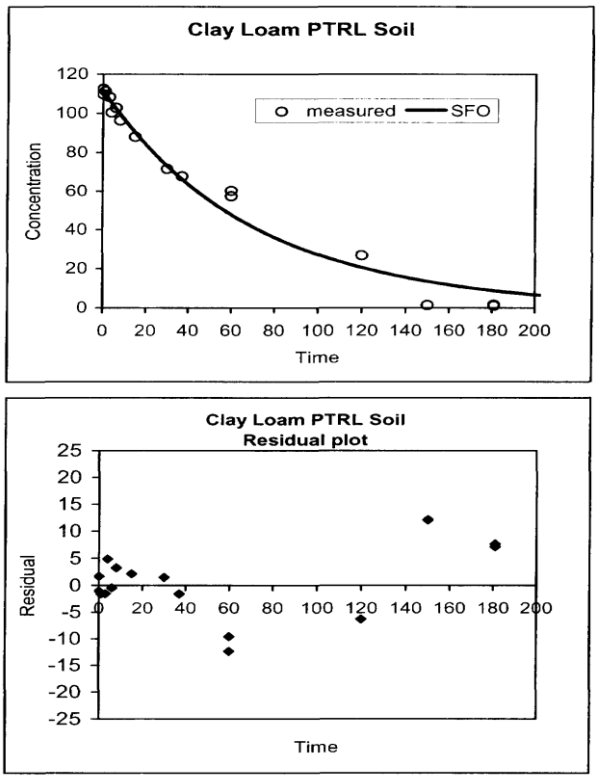


Fig B.8.1.2.3-02 Degradation of N-methyl-bentazone in soil incubated under aerobic condition at 20°C, PTRL soil.



A summary of the estimated persistence (original half-lives) and modelling laboratory half-lives which are normalised to reference conditions of 20 °C temperature and moisture at pF2 is given in Table B.8.1.2.3-03.

Table B.8.1.2.3-03 Laboratory half-lives of N-methyl-bentazone at 20 °C and moisture at pF2

Soil	DegT50/DegT90 original [days]	soil type [USDA]	Actual Moisture [%]	Reference moisture (at pF2) [%]	moisture correction factor [-]	DegT50/DegT90 normalized to moisture at pF2 [days]	Source
LUFA 2.2	153.0 / 508.3	Loamy sand	19.4	14	1.00	153.0 / 508.3	II A 7.2.3/1 2005/1026922
LUFA 3A	38.0 / 126.2	Loam	20.5	25	0.87	33.0 / 109.8	
PTRL soil	49.0 / 162.8	Clay loam	18.2	28	0.74	36.2 / 120.4	

Geometric mean	**65.8 / 218.6**					**56.8 / 188.7**	
Median	**49.0 / 162.8**					**36.2 / 120.4**	
CV	**79%**					**92%**	

The degradation rates of N-methyl-bentazone were investigated in three different soils incubated under aerobic conditions at 20 °C over a period of six months (181 days). Kinetic evaluation of obtained experimental results revealed persistence DegT50 endpoints of 38 - 153 days with a geometric mean of 65.8 days and DT_{90}-values between 126 to 508.3 days with a geometric mean of 218.6 days .

The laboratory modelling normalised DegT50 endpoints of N-methyl-bentazone range from 33.0 to 153.0 days with a geometric mean of 56.8 days. The corresponding DegT90 values range from 109.8 to 508.3 days with a geometric mean of 188.7 days.

Comment

The study preceeds the publications of FOCUS Kinetics guidance. Fitting was roughly done in line with the guidance. Fitting parameters were derived and reported and visual fits were presented. Using this approach endpoints for fate modelling are derived. The report only provides χ^2 for the fits, no statistical paramenters were provided, no t-prob. was reported. The results are used for risk assessment.

Second notifier data

STUDY IIA, 7.2.3/02

Characteristics

reference	:	Traub, M., 2012	study type	:	aerobic degradation
year of execution	:	2011	incubation time	:	up to 120 d
GLP statement	:	Yes	nominal concentration	:	1 mg/kg
guideline	:	OECD 307 (2002)	temperature	:	20°C
test substance	:	[N-methyl bentazone.	DT50	:	see results
purity	:	chemical purity 94.09, (batch no. AFYC110701)		:	
soils	:	loam, sand, loamy sand and	acceptability	:	**acceptable**

		sandy loam	Previous evaluation	:	Submitted for renewal (essential)

Study design

For test soils (soil properties see Table below) fresh batches from LUFA Speyer were used. Each soil was adjusted to 45% MWHC and pre-incubated at 20°C for 6 days to stimulate microbial activity. The application amount for N-Methyl Bentazone was 1.00 mg/kg soil which corresponds to 100 µg/100 g dry soil. Samples were incubated under a continuous humid air supply in the dark at 20°C for up to 120 days. Samples were taken at 0, 3, 7, 14, 30, 63 and 120 DAT. The soil moisture content was maintained at 45% MWHC throughout incubation.

Table B.8.1.2.1-01 Properties of study soils

Parameter	**LUFA 2.2**	**LUFA 2.3**	**LUFA 2.4**
% Sand/silt/clay(A)	77.2/16/6.8	63.6/29.8/6.6	31.7/43/25.3
Texture(A)	Sandy loam	Sandy loam	loam
pH ($CaCl_2$)	5.4	7.21	7.49
% organic carbon	1.53	0.79	1.85
CEC (meq/100 g)	8.9	10.5	32.8
MWHC (g/100 g)	46.8	41	48.4
WHC at pF 2 (g/100 g)	15.2	16.2	28.6
Bulk density (disturbed) g/m^3	1.166	1.176	1.188
Microbial biomass (start)	129.6 mg C/100 g	73.4 mg C/100 g	241.6 mg C/100g
Microbial biomass (end)	92.7 mg C/100 g	54.3 mg C/100 g	184.8 mg C/100 g

(A) USDA classification system.

The test item was extracted from the soil with 80 mL acetonitrile/water (4/1, v/v). The extraction was

repeated for one time. Additional one extraction of the samples was done using a microwave. The soil samples were extracted with 80 mL acetonitril/water (4/1, v/v) for 15 minutes at 60-70 °C.

The ambient and microwave extracts were combined for HPLC-MS/MS analysis. The degradation time of the test item was evaluated according to FOCUS Kinetics using the software KinGUI. The initial concentration at 0 d was included in the parameter optimization procedure, but for an optimal fit, the value was allowed to be estimated by the model.

Results

The method validation was conducted according to guideline, During method validation, mean recoveries of N-Methyl Bentazone were 98.7% to 100.7% at 0.05 mg/kg soil, 97.8% to 99.4% at 0.1 mg/kg soil and 100.1% to 100.8% % at 1.0 mg/kg soil. The determined values of the blank samples were less than 20% of the assigned LOQ of the test item. The extraction efficiency during the study was demonstrated by recovery samples. Therefore untreated samples of each soil were fortified with N-Methyl

Bentazone at 5% of the application rate (= LOQ) and with the same amount as the treated flasks, whereas the mean recoveries were between 94.7-100.3%.

The biomass during the study was rather constant. Results of biomass measurements are reported in table B.8.1.2.1-02.

Table B.8.1.2.1-02 Biomass of soil

		Soil 2.2	Soil 2.3	Soil 2.4
Date of sampling	Days after application [d]	Biomass of untreated samples [mg C/100 g]		
19/10/2011	-12	129.6	57.1	226.8
31/10/2011	0	141.7	73.4	241.6
02/01/2012	60	114.8	46.8	223.4
28/02/2012	120	92.7	54.3	184.8

In tables B.8.1.2.1-03 the results of the degradation of N-Methylbentazone are reported.

Table B.8.1.2.1-03 Degradation of N-Methyl Bentazone

Soil/Fraction	**Percentage remaining at incubation time (days)**						
	0	**3**	**7**	**14**	**30**	**63**	**120**
2.2							
N-methyl bentazone	98.8±2.3	92.6±4.0	97.0±3.7	91.8±2.7	78.8±2.7	54.4±1.4	40.7±8.8
2.3							
N-methyl	99.9±2.6	98.3±4.8	91.1±0.4	86.0±3.9	75.1±1.3	40.3±10.3	14.8±0.8

Soil/Fraction	Percentage remaining at incubation time (days)						
	0	**3**	**7**	**14**	**30**	**63**	**120**
bentazone							
2.4							
N-methyl bentazone	92.9±2.5	90.2±2.5	88.7±2.7	79.8±4.8	68.2±0.4	37.6±2.6	3.3±5.3

The rate of degradation of N-methyl bentazone in soil was analyzed by nonlinear regression of the original data by applying first order kinetics.

Table B.8.1.2.1-04 Degradation rate constant calculations (20 ºC) Properties of study soils

Soil	Trigger values				
	DT_{50} (d)	**DT_{90} (d)**	**Model**	**r^2**	**χ^2error (%)**
2.2	86	286	SFO	0.964	2.58
2.3	50	165	SFO	0.981	3.26
2.4	44	146	SFO	0.968	2.80

In figure B.8.1.2.1-01 to B.8.1.2.1-02 the visual fits of the three soils are presented.

Figure B.8.1.2.1-01 1st order fit of concentration versus time of N-Methyl Bentazone in soil LUFA 2.2

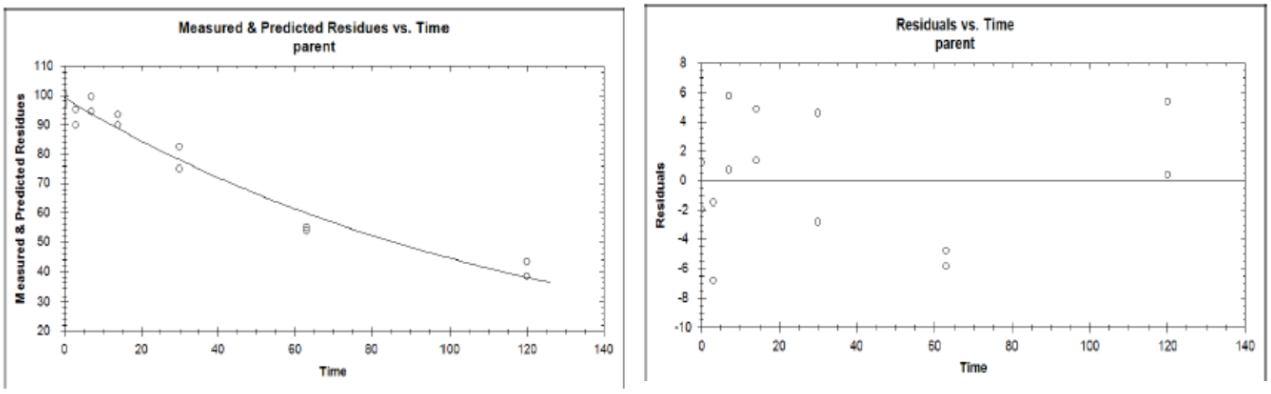


Figure B.8.1.2.1-02 1st order fit of concentration versus time of N-Methyl Bentazone in soil LUFA 2.3

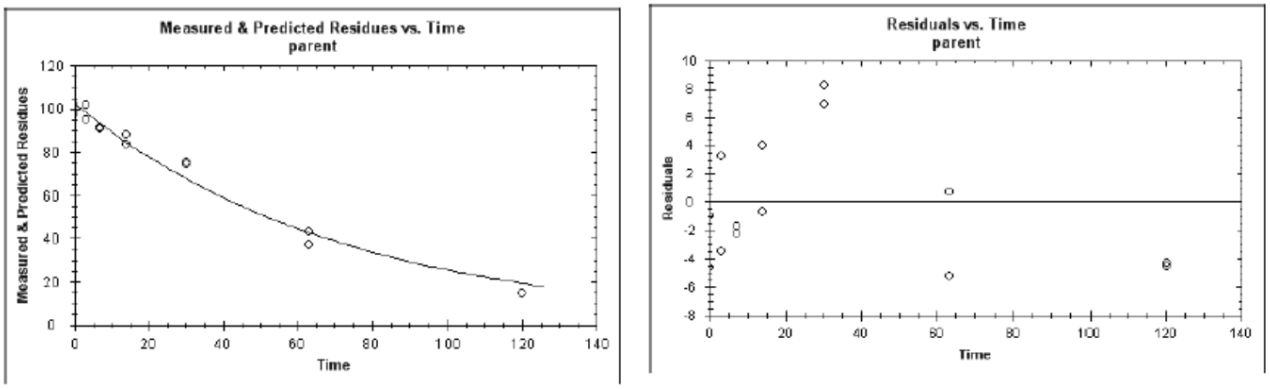
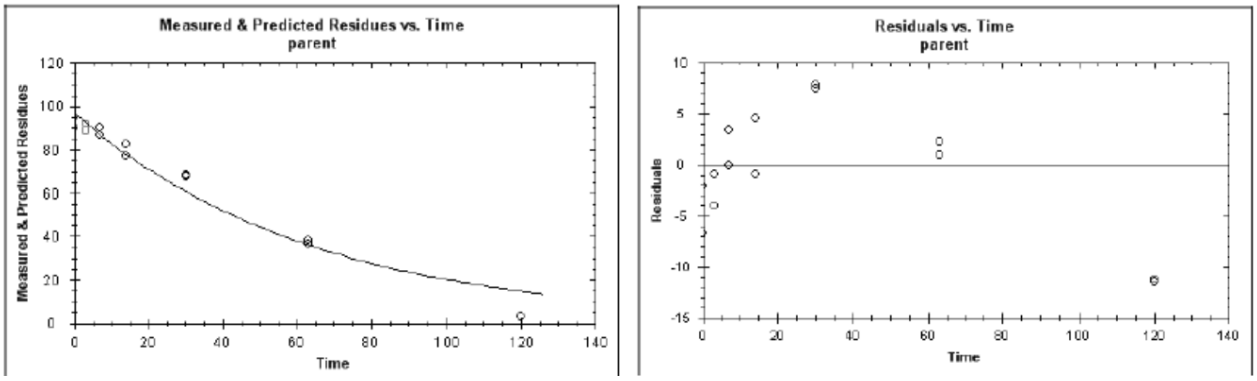


Figure B.8.1.2.1-03 1st order fit of concentration versus time of N-Methyl Bentazone in soil LUFA 2.4



Comments

The authors reported that the analysis of degradation was done according to FOCUS Kinetics. The SFO fit was done using the software model KINGUI and fitting parameters were derived and reported. Using this approach endpoints for fate modelling are derived. The report only provides χ^2 for the fits, no statistical paramenters were provided, no t-prob. was reported. The results are considered for risk assessment.

During the peer review process the values were normalised for reference conditions. The values are included in the table below.

Soil	**DegT50/DegT90 original [days]**	**soil type [USDA]**	**Actual Moisture [%]**	**Reference moisture (at pF2) [%]**	**moisture correction factor [-]**	**DegT50/DegT90 normalized to moisture at pF2 [days]**

LUFA 2.2	86	286	Sandy loam	21.06	15.2	1.00	86 286
LUFA 2.3	50	165	Sandy Loam	18.45	16.2	1.00	50 165
LUFA 2.4	44	146	loam	21.78	28.6	0.826	38.4 120.6
Geometric mean							**54.9 / 178.5**

STUDY IIA, 7.2.3/03

Characteristics

reference	:	Verhaar. H.J.M., 2012 (statement)	study type	:	Statement
year of execution	:	2012	incubation time	:	-
GLP statement	:	Yes (GMP)	nominal concentration	:	-
guideline	:	Not applicable	temperature	:	-
test substance	:	Not applicable	DT50	:	see results
purity	:	Not applicable		:	
soils	:	Not applicable	acceptability	:	**Not acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Study design

Soil degradation data for N-methyl bentazone (study IIA 7.2.3/02), determined under laboratory conditions, were reanalyzed according to the FOCUS Degradation Kinetics Guidance Document (Sanco/ 10058/2005, v.2.0). The analysis of degradation was done according to FOCUS Kinetics in the study. For use in soil degradation modelling, these DT50 values need to be normalised to standard conditions according to FOCUS Guidance (as incorporated in the DT50Convert_FOCUS Excel spreadsheet as prepared by M. Vanclooster).

Results

The normalisation to standard conditions results in the following DT50 values:

Table B.8.1.2.1-05 Degradation rate constant calculations

	DT50 from study	Normalized DT50
LUFA Speyer 2.2 soil	86	123
LUFA Speyer 2.3 soil	50	64
LUFA Speyer 2.4 soil	44	43.6

Comment

The calculations of the standardisation to normalised conditions are not provided in the statement. It is only referred to a spreadsheet that is not (made) available to RMS. As it cannot be verified if the standardisation to reference conditions was done correctly the results cannot be accepted. The original study was done at 20ºC and 45% of MWHC. This is according to guidance on soil degradation. Therefore it seems unrealistic that the normalised values are substantially higher. The original study is partly acceptable (persistence assessment), those results of the original study will be used.

B.8.1.4 Summary route and rate of degradation in soil

Route of degradation

Overall summary on metabolic pathway in soil.

Bentazone degradation in soil is mainly characterized by incorporation into the organic soil structure with tight binding to fulvic acids, humic acids and humines.

The overall understanding on the route of degradation of bentazone in soil was confirmed by the studies performed according to the newest guidelines and analytical techniques. The major route of degradation in soil is formation of bound residues with tight incorporation into all fractions of the organic soil matrix (up to 64% of the applied radioactivity distributed between fulvic acids, humic acids and humins after 120 days). First metabolisation step is assumed to be hydroxylation and the phenyl-moiety. The resulting 6- or 8-OH-bentazones are then quickly incorporated into the humic substances. Mineralization reached 8% within 120 days.

Minor pathways were the methylation and halogenation (prevailing chlorination, but also bromination) of the active ingredient. Both reactions are known to be performed by soil microorganisms. All those metabolites occurred only in minor amounts in soil. Since N-methyl-bentazone was the only metabolite which slightly exceeded 5% of applied radioactivity in two consecutive soil samplings see also chapter 7.2 Rate of Degradation), it is considered for further environmental risk assessments according to new guidance documents introduced since last Annex I inclusion.

No other peak exceeded 4% in any of the new bentazone studies (including soil photolysis). Halogenated bentazone derivatives were not identified. If formed at all, they were among those minor peaks < 4% AR.

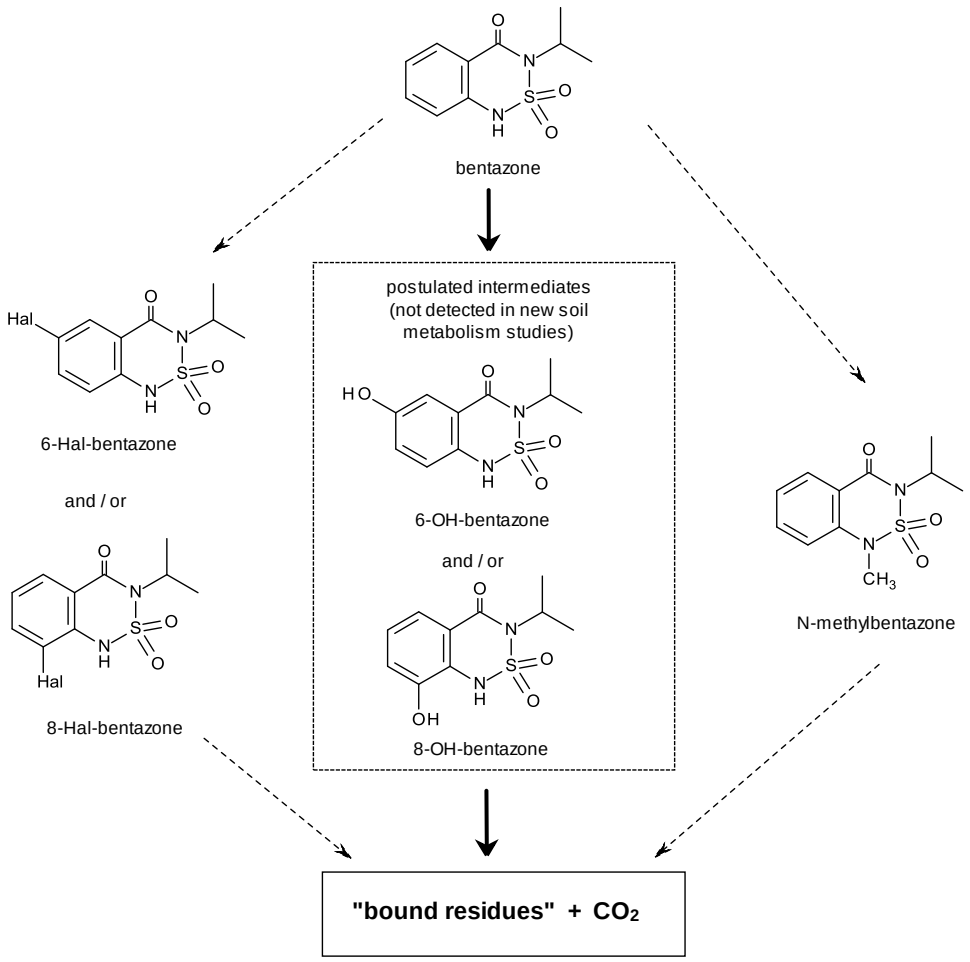
The degradation is considerably slowed down under anaerobic conditions. The new soil photolysis study showed a faster degradation under irradiated conditions compared to dark conditions, however, no new metabolites appeared. It can be concluded that the route of degradation is still the same under sunlight, only the formation rate of bound residues seems to be enhanced.

In the original DAR, soil photolysis was shown to be no significant degradation route of bentazone with an almost unchanged concentration of bentazone in soil after about 39 days exposure. In a non acceptable study of de Vette (2002) showed increasd degradation. A decrease in extractable residue was observed from 94.6% of initial activity at the start of the test to 32.8% after 32 days. The amount of radiolabelled carbon dioxide increased to 26.8% of the initial radioactivity after 32 days of incubation. A more recent soil photolysis study submitted with the AIRII dossier however showed a slightly increased degradation rate under irradiated conditions, leading to an enhanced incorporation into humic substances. The concentration of bentazone decreased from 98.2% TAR at the beginning to 48.7 % TAR in the course of the photolysis study. Metabolites remained < 5% of applied radioactivity. The half-live values (SFO DT_{50}) for bentazone in the test systems were calculated to be 12.8 days under continuous irradiation and 42.1 days in the dark.

Leaching evaluation and exposure assessments for ecotoxicological risk assessments according to current guidelines and guidance documents were therefore performed for the active ingredient bentazone and its metabolite N-methyl-bentazone.

An updated route of degradation of bentazone in soil in shown in Figure 7.1

Fig 7.1 Proposed pathway for the degradation of bentazone in soil (update 2012)



Hal = halogenated bentazone (Cl or Br, only detected in older studies)

Summary: Rate of degradation in laboratory studies

A summary of already peer-reviewed DT50 values from the previous Annex I evaluation as listed in the EU review report (2000) is given below. The degradation rates of bentazone under laboratory conditions showed a rather high variability. DT_{50} values ranged from 8 to 102 d with an average of 45 days according to the EU review report.

Soil no.	..appl. rate mg/kg	pH	Moisture %	Temperature °C	$DT50_X$ d	DT90x d	Ref .
1	2	6.7	15	22 +/- 2	12	39	(1)

1	5	6.7	15	22 +/- 2	25	-	(1)
Influence of moisture							
1	2	6.7	5	22 +/- 2	20	66	(1)
1	2	6.7	10	22 +/- 2	18	59	(1)
1	5	6.7	15	22 +/- 2	23	76	(1)
Influence of temperature							
1	2	6.7	12	8 - 10	161**)	-	(1)
1	2	6.7	12	22 +/- 2	35**)	-	(1)
1	2	6.7	12	35 - 37	35**)	-	(1)
Influence of pH							
4	2	6.4	12	22 +/- 2	34	113	(1)
4	2	4.6/5.5	12	22 +/- 2	10	33	(1)
2…..2		7.5	20	22 +/- 2	56	-	(1)
2…..3/3		7.5	20	22 +/- 2	70/84	-	(3)
3…..2		5.1	20	22 +/- 2	102	-	(1)
4……3/3/3		4.6	12	22 +/- 2	42/46/47	-	(2)
5……10		6.1	52	22 +/- 2	65	215	(4)
6……10		5.0	31	22 +/- 2	45	151	(4)
7……10		7.7	61	22 +/- 2	45	150	(4)
9……5		6.8	40	20	38	125	(5)
10…..5		5.2	40	20	16	54	(5)
11…..5		7.2	40	20	11	37	(6)
12…..5		6.7	40	20	47	198 (sandy)	(6)
13…..2		7.1	40	20	80	-	(6)
14…2		5.8	40	20	8	85	(6)

*) All calculation were conducted according (7)(8) if not stated otherwise.

**) Graphical interpolation

The basis of the studies and the selection of mean parameters are the following references:

1. Drescher and Otto (1972). Studies on the degradation of bentazone (BAS 351-H) in soil. BASF DocID 1972/0030.
2. Drescher and Otto (1973). Studies on the degradation of bentazone (BAS 351-H) in soil – 2nd report. BASF DocID 1973/0031.

3. Drescher and Otto (1973). Über den Abbau von Bentazon im Boden (4. Mitteilung). BASF DocID 1973/0047.
4. Anonym (1974). Verhalten des Pflanzenschutzmittelwirkstoffes im Boden. BASF DocID 1974/10086.
5. Keller, E. (1987). The aerobic soil metabolism of BAS 351 H (Bentazone). BASF DocID 1987/0415.
6. Keller, W. (1988). Degradation behavior of Bentazone in soil. BASF DocID 1988/10121.

For several reasons mentioned under B.8.1.2.1 the information on rate of degradation from the original DAR is not considered reliable enough for current risk assessment and are reported for information only. Only those studies carried out according to a relevant guideline (e.g. OECD or SETAC) were used for the following overview. All other studies are considered as supplemental information and were not included in the selection of the laboratory degradation endpoints. The selection of endpoint is described below.

Main notifier data

A summary of all estimated persistence (original half-lives) and modelling laboratory half-lives which are normalised to reference conditions of 20 °C temperature and moisture at pF2 is given in Table B.8.1.4-01 for the parent compound bentazone.

B.8.1.4-01 Laboratory half-lives of bentazone at 20 °C and moisture at pF2

Soil	DegT50/DegT90 original [days]	soil type [USDA]	Actual Moisture [%]	Reference moisture (at pF2) [%]	moisture correction factor [-]	DegT50/DegT90 normalized to moisture at pF2 [days]	Source
Bruch West	45.1/ 149.8	sandy loam	9.44	19	0.613	27.6/ 91.8	II A 7.1.1/01 2010/1057318
Bruch West	33.0/ 109.6	sandy loam	11.28	19	0.694	22.9/ 76.1	II A 7.2.1/1 2011/1000621
Li10	43.4/ 144.2	loamy sand	10.28	14	0.806	35.0/ 116.1	

LUFA 2.2	30.9/ 102.6	loamy sand	13.52	14	0.976	30.2/ 100.2	
LUFA 2.3	49.1/ 163.1	sandy loam	10	19	0.638	31.3/ 104.1	
Borstel	16.9/ 56.1	loamy sand	11.6	14	0.877	14.8/ 49.2	Ebert, 2000 2000/1000142
Geometric mean	**30.1/ 114.3**					**26.0/ 86.4**	
Median	**32.0/ 126.9**					**28.9/ 96**	
CV	**49%**					**45%**	

Second notifier data

A summary of original half-live estimated from study IIA, 7.1.1-06 (persistence) is given in the table below .

B.8.1.4-02 Laboratory half-lives of bentazone (20°C) not normalised for moisture

Soil	**Trigger values**				
	DT_{50} (d)	**DT_{90} (d)**	**Model**	**r^2**	**χ^2error (%)**
Loamy sand	9.6	32	SFO	0.964	2.58

B.8.1.4-02b Laboratory half-lives of bentazone (20°C) normalised for moisture

Soil	**DegT50 original [days]**	**soil type [USDA]**	**Actual Moisture [%]**	**Reference moisture (at pF2) [%]**	**moisture correction factor [-]**	**DegT50 normalized to moisture at pF2 [days]**
Borstel	9.6	Loamy sand	30.94	24	1	9.6

4.1.4.3 Photolytic degradation

4.1.4.3.1 Aqueous photolysis

Main notifier data

STUDY IIA, 7.6/01

Characteristics

reference	:	Singh M., 2011(a)	incubation time	:	up to 15 d
study type	:	aqueous photolysis	nominal concentration	:	17.8 mg/L (pH 5 and 7); 16.7 mg/L (pH 9)
year of execution	:	2009	pH/temperature	:	pH 5,7,9/temp 22°C
GLP statement	:	yes	light intensity	:	618 W/m^2
guideline	:	OECD 316 EPA 161-2;EPA 835 2240	Conclusion	:	See results
test substance	:	^{14}C-bentazone (BAS 351H), phenyl label Batch# 210-2201	acceptability	:	**acceptable**
purity	:	(1) Chemical purity not reported, radiochemical purity 97.3%.	Previous evaluation	:	Submitted for renewal (essential)

Study design

The aqueous photolysis of ^{14}C-BAS 351 H (^{14}C-bentazone, phenyl label) was investigated in buffer solutions pH 5, 7 and 9 at a temperature of 22 ± 1°C. Sodium acetate was used for the preparation of pH 5 buffer solution. TRIS (hydrochloride) and TRIS (base) were used for the preparation of pH 7 and pH 9 test buffer solutions (0.01 M), respectively. The buffer solutions were autoclaved at 120°C before use.

The treated solutions were continuously exposed to artificial sunlight (Xenon lamp with filters to cut-off wavelengths <290 nm) in an Atlas Suntest CPS Plus apparatus for about 15 days. The average light intensity in the wavelength range between 300 - 800 nm was measured to be 618 W/m2, comparable to natural sunlight at 40° N latitude.

During irradiation, CO_2-free sterile air was purged over the samples and through the NaOH traps. Dark control samples in HPLC vials consisting of 1mL buffer aliquots (pH 5, 7 and 9) treated with ^{14}C-phenyl-labeled BAS 351 H were stored in a dark incubator maintained at 22 ± 1°C.

Duplicate treated samples, (irradiated and dark control) were analysed concurrently by LSC and HPLC after about 0, 3 (4), 6, 10 (11), 13, and 15 days after treatment (DAT).

For the isolation and identification of the ^{14}C degradation products aliquots of the photolysis samples were analysed by LC/MS/MS.

For determination of the quantum yield of BAS 351 H, a mixture of p-nitroacetophenone (PNAP, 2.6 x 10^{-5} M) and pyridine (2 x 10-2 M) was used as chemical actinometer. The vessel with the actinometer solution

was irradiated under similar conditions as the other test vessels. Sterility checks were performed for the experimental and actinometer solutions at starting and end points of incubation. The test systems were under sterile conditions for the entire experimental period.

Results

The recovery of radioactivity in the irradiated and dark control samples as well as the amount of radioactivity in the volatile traps is shown in Table B.8.4.2-01. The material balance for the irradiated test systems ranged from 92.8–101% TAR, 93.8-100% TAR and 94.4-100% TAR for pH 5, pH 7 and pH 9 buffer, respectively. The material balance for the dark controls ranged from 97.9 – 101% TAR, 96.8–100% TAR and 99.8-102% TAR for pH 5, pH 7 and pH 9 buffer, respectively. The volatile radioactivity increased with time for all the test systems.

Table B.8.4.2-01 Recovery of radioactivity during aqueous photolysis of [U-^{14}C-phenyl]-BAS 351 H; mean of two replicates [% TAR]

days after treatment	pH 5 Cumulative Volatile	pH 5 Material balance	pH 7 Cumulative Volatile	pH 7 Material balance	pH 9 Cumulative Volatile	pH 9 Material balance
irradiated						
0		100		99.5		99.9
3/4	1.0	97.9	0.65	96.4	0.21	99.3
6	2.7	97.3	1.9	96.9	0.54	98.9
10/11	6.2	94.4	3.7	95.0	1.9	97.9
13	9.0	93.6	5.2	94.2	2.7	95.6
15	11.1	93.6	6.3	94.3	3.5	97.2
dark control						
0	-	100	-	99.5	-	99.9
3/4	-	98.3	-	97.2	-	101
6	-	99.3	-	97.7	-	101
10/11	-	98.6	-	98.1	-	101
13	-	99.6	-	98.3	-	101
15	-	99.2	-	97.8	-	100

In all irradiated test sytems, BAS 351 H degraded to a number of products. After 15 days continuous irradiation, a total of 15 radioactive residues could be detected by HPLC in the sample of the pH 5 buffer solution. Bentazone, PeakB, and PeakC (sulfonic acid) were found to be the major radioactive residues. The amount of bentazone decreased with time from 97.9% TAR to 4.2 %TAR at the end of the study. The degradation product PeakB increased with time and accumulated to 26.3% TAR by the final sampling interval. The degradation product PeakC (sulfonic acid) also increased over time and accounted for 20.2%

TAR at 15 DAT. The degradation product UNK 2.6 reached a maximum amount of 5.7%TAR. This peak was isolated and further characterized during the pH 9 photolysis experiment and could be shown to be a mixture of multiple components. The degradation product listed as "Others" was a mixture of multiple components and none of the individual components exceeded 4.8%TAR. One of these components was identified as BH 351 AIPAM. The analytical results of the pH 7 buffer revealed a total of 17 radioactive residues. Bentazon, PeakB, PeakC (sulfonic acid) and UNK 2.5 were found to be the major radioactive residues during the study. The amount of bentazon decreased with time from 96.0 %TAR to 14.4 %TAR at the end of study. The degradation product PeakB increased with time and accumulated to a maximum of 16.8% TAR by the final sampling interval. The degradation product PeakC (sulfonic acid) also increased over time and accounted for a maximum of 25.2% TAR at 13 DAT. The very polar degradation product UNK 2.5, increased to 10.0% TAR at 15 DAT. "Others" at pH 7 was a mixture of multiple components with none of the individual components exceeded 4.9%TAR. Also here BH 351 AIPAM was identified as one of these. In the pH 9 buffer system a total of 17 radioactive residues was identified. Bentazone, PeakB, PeakC (sulfonic acid), UNK 17.5 and UNK 2.5 min were found to be the major radioactive residues during the study duration. The amount of bentazone decreased with time from 97.7 %TAR to 8.8 %TAR at the end of the study. The degradation product PeakB slowly increased over time and accumulated to a maximum of 8.5% TAR at the end. The degradation product PeakC (sulfonic acid) also increased to a maximum of 23.7% TAR at 10 DAT and then began to decline slowly (15 DAT: 22.8 % TAR). The degradation product UNK 17.5 accumulated to a maximum of 9.9% TAR (10 DAT) and then began to decline. This degradation product was found to be a mixture of several components. The very polar degradation product UNK 2.5 increased to about 29.5% TAR at 15 DAT. This degradation product was found at pH 7 and 9 only and is a mixture of several components. The degradation product listed as "Others" was a mixture of multiple components and none of the individual components exceeded 4.8%TAR. UNK 2.5, found at pH 5 only, also consisting of a mixture of components just reached 5% after 15 days.

The HPLC analysis of the dark control revealed only bentazone as major radioactive residue at every sampling interval. The amount ranged from 95.2 to 97.9%, 94.5 to 96.0% and 97.7 to 99.5% TAR for pH 5, pH 7 and pH 9 test systems, respectively.

Table B.8.4.2-02 HPLC quantitation of ^{14}C-residues in the dark controls treated with [U-^{14}C-phenyl]-BAS 351 H; mean of two replicates [% TAR]

	pH 5		**pH 7**		**pH 9**	
	BAS 351 H t_R ~18.3 min	**Others[1]**	**BAS 351 H t_R ~18.3 min**	**Others[1]**	**BAS 351 H t_R ~18.3 min**	**Others[1]**
0	97.9	2.5	96	3.5	97.7	2.2
3	95.3	3.1	94.6	2.6	99.3	1.6
6	95.8	3.6	94.6	3.2	98.8	2.0

	pH 5		pH 7		pH 9	
	BAS 351 H t_R ~18.3 min	Others[1]	BAS 351 H t_R ~18.3 min	Others[1]	BAS 351 H t_R ~18.3 min	Others[1]
10	95.2	3.4	95	3.2	99.2	1.9
13	96.1	3.6	95.3	3.1	99.3	2.0
15	96.4	3.0	94.5	3.4	99.5	1.0

[1] Sum of multiple components, none totalling more than 1.3% TAR

t_R = retention time

Table B.8.4.2-03 HPLC quantitation of ^{14}C-residues in the irradiated test system pH 5 treated with [U-14C-phenyl]-BAS 351 H; mean of two replicates [% TAR]

DAT	UNK 2.6[1] (t_R ~ 2.6 min)	UNK 7.3 (t_R ~ 7.3 min)	Peak B (t_R ~ 8.2 min)	Peak C (t_R ~ 15.8 min)	BAS 351 H t_R ~18.3 min	Others[2]
0	-	-	-	-	97.9	2.4
3	-	1.3	13.7	8.7	67.9	5.3
6	-	3.2	24.4	14.7	41.4	10.9
10	3.0	4.4	29.9	19.7	17.0	14.3
13	4.9	5.6	29.7	21.1	7.5	16.0
15	5.7	5.7	26.3	20.2	4.2	20.5

- not detectable

[1] 2.6 minute peak was very polar and a mixture of several components

[2] Sum of multiple components, no single peak exceeds 4.8% TAR

t_R = retention time

Table B.8.4.2-04 HPLC quantitation of ^{14}C-residues in the irradiated test system pH 7 treated with [U-^{14}C-phenyl]-BAS 351 H; mean of two replicates [% TAR]

DAT	UNK 2.5[1] (t_R ~ 2.5 min)	Peak B (t_R ~ 8.2 min)	Peak C (t_R ~ 15.8 min)	BAS 351 H t_R ~18.3 min	Others[2]
0	-	-	-	96	3.5
3	1.4	4.3	8.2	77.7	4.2
6	3.0	9.8	15.3	57.6	8.9
10	6.6	14.3	23.0	32.1	15.5
13	7.9	16.7	25.2	20.6	18.8
15	10.0	16.8	25.0	14.4	21.8

- not detectable

[1] 2.5 minute peak is very polar and a mixture of several components

[2] Sum of multiple components, no single peak exceeds 4.8% TAR

t_R = retention time

Table B.8.4.2-05 HPLC quantitation of ^{14}C-residues in the irradiated test system pH 9 treated with [U-^{14}C-phenyl]-BAS 351 H; mean of two replicates [% TAR]

DAT	UNK 2.5[1] (t_R ~ 2.5 min)	UNK 3.5 (t_R ~ 3.5 min)	Peak B (t_R ~ 8.2 min)	Peak C (t_R ~ 15.8 min)	UNK 17.5[1] (t_R ~ 17.5 min)	BAS 351 H t_R ~18.3 min	Others[2]
0	-	-	-	-	-	97.7	2.2
3	8.8	0.7	3.6	12.7	4.9	65.9	2.4
6	14.0	1.6	5.3	19.2	8.2	44.7	5.7
10	24.7	6.1	7.6	23.7	9.9	14.8	9.5
13	27.5	5.2	7.5	21.7	8.5	10.3	12.5
15	29.5	5.1	8.5	22.8	8.2	8.8	10.9

- not detectable

[1] Peaks at ~2.5 minutes and ~17.5 minutes were a sum of multiple components

[2] Sum of multiple components, no single peak exceeds 4.8% TAR

Estimated photolytic half-lives and quantum yields are given in Table B.8.4.2-06

Table B.8.4.2-06 Photolytic half-lives and quantum yield of BAS 351 H in sterile buffer

pH of buffer	DT50 [d]	Data Correlation coefficient	Quantum Yield (mol Einstein-1)
pH 5	3.3	0.977	7.7 x 10-3
pH 7	5.4	0.965	4.7 x 10-3
pH 9	3.9	0.968	6.0 x 10-3

The theoretical half-lives of BAS 351 H in the top layer of natural aqueous systems were calculated with a program which uses the algorithms developed by Frank and Klöpffer (*Frank, R, and Klöpffer, W., Ermittlung von Strahlungsdaten und Entwicklung eines Programms zur Abschätzung der abiotischen Transformation von Chemikalien in natürlichen Gewässern, Forschungsbericht Nr. 106 020 46, 1985.*) for the direct phototransformation of chemicals in water. This model uses data specific to the test substance, including the quantum yield (Φ_{ts}), and the absorption coefficient ($\varepsilon_{(\lambda)ts}$). The model also uses natural light intensities measured for each month in central Europe. Other inputs to the model included specifying a 1 cm thickness of the upper layer of water, a 10 mg/L (or 10 ppm) water concentration, and 10% losses by reflection. The DT_{50} calculation was made for each month from January to December.

Table B.8.4.2-07 Theoretical photolytical half-lives of BAS 351 H in the top layer of aqueous systems

Month of application	Day length [h]	Theoretical environmental half-life					
		pH 5		pH 7		pH 9	
		DT_{50} [h]	DT_{50} [d]	DT_{50} [h]	DT_{50} [d]	DT_{50} [h]	DT_{50} [d]
April	13.67	1.46	0.11	2.41	0.18	1.76	0.13
May	15.44	1.27	0.08	2.08	0.13	1.53	0.10
June	16.47	1.20	0.07	1.97	0.12	1.44	0.09
July	16.07	1.32	0.08	2.18	0.14	1.59	0.10
August	14.53	1.25	0.09	2.06	0.14	1.51	0.10
September	12.56	1.91	0.15	3.14	0.25	2.30	0.18
October	10.57	3.14	0.30	5.16	0.49	3.77	0.36
November	8.65	6.32	0.73	10.39	1.20	7.60	0.88
December	7.55	11.05	1.46	18.16	2.41	13.28	1.76
January	8.02	7.14	0.89	11.73	1.46	8.58	1.07
February	9.67	3.85	0.40	6.33	0.65	4.63	0.48
March	11.60	2.18	0.19	3.59	0.31	2.63	0.23

The results showed that bentazone was rapidly degraded in sterile water under photolytic conditions. The degradation half-lives were about 3.3 days, 5.4 days and 3.9 days in pH 5, 7 and 9 buffer solutions, respectively. Bentazone was stable in test buffers (pH 5, 7 and 9) under dark conditions. A rather large number of photo-products was formed (≥ 15), two of them exceeding 10% of the total applied radioactivity. These two peaks were designated as PeakB (max. 30% TAR at pH 5), and PeakC (max. 25% TAR at pH 7). The rest of the degradation products were minor and did not exceeded 5% TAR.

Comment

During the peer review a data requirement was set for the identification of all photolysis metabolites occurring >10%. In the study by Singh these were reported as PkB and PkC. Identification of these metabolites was done by Ellenson (1989), already part of the original dossier of bentazone but not included in the original DAR, and Singh (2009) as reported under 2.9. Ellenson elucidated peak C to be 1-[N-methylethyl]-1-sulfoamino-benzamide by HPLC. Further work on peak B (NMR; LC-MS methods) identified it as 3-isopropyl-2,3-dioxo-5-oxocyclopenteno [d]-1 H-2,1,3-thiadiazine-4(3H)-one-6-carbonic acid). In the more recent study by Singh, PkC was identified to be sodium 2-[(isopropyl-amino)-carbonyl] phenylsulfamate (M351H024)

Other published studies

STUDY IIA, 7.6/02

Characteristics

reference	:	Housari F.A. et al. (2010a)	Study type	:	Hydrolysis and photodegradation in water
year of execution	:	2010	Incubation time	:	8 d
GLP statement	:	No	Nominal concentration	:	50 µM
guideline	:	None	temperature	:	Not reported
test substance	:	Bentazone.	DT50		See results
purity	:	purity 99.7%			
			acceptability	:	**additional**
			Previous evaluation	:	Submitted for renewal (supplementary)

Method

The dissipation of three acidic herbicides, among them bentazone, was investigated in water from the Vaccarès lagoon (brackish water) and the Canal de Fumemorte (fresh water) in the Camargue region. They differ mainly in the type and quantity of organic matter, and in the salinity, which is below 1 g/L in the canal water and about 25 g/L in the Vaccarès lagoon. Two different types of water samples were selected to elucidate if there is any or possible impact of water type on photo-degradation. Water samples were taken from about 15 cm below the surface (lagoon and canal), filtered through 0.45 µm pore-size membranes, and stored at 4 °C in the dark till analysis. Storage and all measurements were performed at the pH of the natural water. The quantification of bentazone was done directly by HPLC chromatograph equipped with a UV detector (215 nm)and using a C-18 column. The limit of detection was 25 ± 3 µg/L. Three blank samples were obtained by spiking ultra-pure water with the studied herbicides to give final concentrations of 10 µg/L. Blanks were analysed as a control for memory effect in the instrument and for laboratory contamination. The average recoveries ranged from 83 to 90% for bentazone with relative standard deviations of 3-4%. All results were corrected with the corresponding recovery rates to provide accurate amounts.

For photo-degradation the samples were exposed to natural sunlight under summer conditions in Marseille, France at a bentazone concentration of 50 µM and over study duration of eight days. Also, aqueous solutions of bentazone at 50 µM in ultra-pure water in tubes were wrapped in aluminium foil and kept in the dark to test for hydrolysis of the herbicide.

Results

The chloride ion content was 1.18 ± 0.03 x 10^{-3} M and 1.90 ± 0.05 x 10^{-1} M in canal water and lagoon water samples, respectively. Non Purgeable Organic Carbon (NPOC) values were 19.8 ± 0.3 and 32.1 ± 0.6 mg C L^{-1} in canal water and lagoon water samples, respectively.

The loss of bentazone was described as a sum of different first order kinetic processes as plots of (In [bentazone]/[bentazone]$_o$) versus time and were found to be linear. The observable rate constant of herbicide degradation is defined as:

$k_{obs} = k_{dp} + k_{ip} + k_{hyd}$

which includes the first-order decay constant of direct k_{dp} (d^{-1}) and indirect photolysis k_{ip} (d^{-1}) and the first order decay constant of hydrolysis k_{hyd} (d^{-1}). The rate constants found for the Vaccarès lagoon water are given in Table B.8.4.2-07.

Table B.8.4.2-07 Summary of elimination rate constants for herbicides in the Vaccarès lagoon.

Elimination process	**Elimination rate [d^{-1}]**
Hydrolysis	k_{hyd} 0.001 ± 1 x 10^{-4}
Direct photolysis	k_{dp} 0.14 ± 2 x 10^{-2}
Indirect photolysis	k_{ip} 0.03 ± 1 x 10^{-2}

The overall half-life times for bentazone were 2.17 ± 0.25 and 4.08 ± 0.32 d for canal and lagoon water samples, respectively. In ultra-pure water incubated in the dark, hydrolysis of bentazone was found to be negligible. The half-life time for bentazone in ultra-pure water under natural sunlight was measured to be 5.12 ± 0.7 d.

The light conditions for the photo-degradation experiments in glass vials differ from those naturally occurring in the lagoon which has an average depth of approximately 1 m and a natural turbidity. Using the model MASAS (Modelling of Anthropogenic Substances in Aquatic Systems) the overestimation of degradation rates under direct exposure was found to be by a factor of 2.5 to 3. The resulting photolytic half-life of bentazone under natural conditions would then be 12 d. The authors concluded that direct and indirect photo-degradation of bentazone are main processes of dissipation in lagoon water. Half-lives of photo-degradation of bentazone were found to be 2.2 and 4.1 days under experimental conditions in canal and lagoon water, corresponding to an estimated half-life of 12 days in lagoon water in the field.

Second notifier data

STUDY IIA, 7.6/03

Characteristics

reference	:	Dawson, I., R. Lynn & G.Y.	incubation time	:	up to 168h

		McCorquodale (2003)			
study type	:	aqueous photolysis	nominal concentration	:	5.0 µg/mL (preliminary study) 5.29 µ/L (main study)
year of execution	:	2002	pH/temperature	:	pH 7 / temp 20±3°C
GLP statement	:	yes	light intensity	:	456 W/m2
guideline	:	Not reported	Conclusion	:	See below
test substance	:	14C-bentazone, phenyl labelled, Batch# CFQ11197 Non labelled Batch No. 245-112B	DT50 DT90		47.8 h 158.8 h
purity	:	Chemical purity non labelled: 99%; radiochemical purity 99.34%.Spec activity 167 MBq/mmol.	acceptability	:	**acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Study design

The objective of this study was to investigate the rate of breakdown of [^{14}C]-Bentazone in pH 7 buffered aqueous solution, during irradiation with artificial sunlight. Samples were irradiated continuously with artificial sunlight of nominal intensity 456 W.m^{-2} from a xenon irradiation source over periods of up to 48 h and up to 168 h for the preliminary and main studies, respectively. The irradiation device was fitted with a u.v. filter to remove light with a wavelength of <290 nm and >800 nm. Vessels were placed under the light source in a cooling bath at 20 ± 3°C. One vessel was incubated at 20 ± 3°C in the dark in the preliminary study only. All vessels were connected to a continuous air-flow system and a stream of moist carbon dioxide-free air was drawn through each vessel for ca 15 min immediately before sampling. The gas mixture leaving each vessel was passed through a series of three traps, the first acting as a safety trap, the second containing ethanediol to trap any liberated organic volatiles and the final trap containing ethanolamine to trap any liberated $^{14}C0_2$. Duplicate exposure vessels were removed for analysis after 0 (control), 12, 24, 48, 96 and 168 h exposure. Following sampling, the amount of radioactivity in each incubate was determined by LSC. [^{14}C]-Bentazone and its photolytic degradation products were characterised by HPLC and TLC.
The formation of 3-isopropyl-2,3-dioxo-5-oxocyclopenteno[d]1H-2,1,3-thiadiazine-4(3H)-one 6 carbonic acid and other breakdown products was further investigated by high performance liquid chromatography (HPLC) and liquid chromatography-mass spectroscopy (LC-MS and LC-MS/MS).

Results

The overall recoveries in the preliminary study were in the range 92.13-99.44%. In the main study the overall recoveries were in the ragen 92.7-99.04%. Levels of radioactivity associated with the ethanediol and ethanolamine traps were low (<1%). HPLC analysis of the main study incubates also indicated that Bentazone rapidly photodegraded to several components. At 0 h in the main study, [^{14}C]-Bentazone quantitatively accounted for the radioactivity present in the samples. As the irradiation period progressed, levels of parent material declined, with a mean of ca 7% of the radioactivity characterised as parent in the study termination samples (168 h). In addition to parent material, up to 7 unknown components were detected. Of the unknown components observed, 4 were present at levels greater, or approximately equal to, 10% of the applied radioactivity, Unknown 6 at a maximum of ca 13% and decreased to ca 8% at 168h. Unknown1 and Unknown4 increased to a max.level of 19% and 24% respecively and hardly decreased thereafter. Unknow 5 reached a max.level of 13% at 168h. Unknown 6 reached a maximum of 10.6% of 48h. Unknown1 was comprised of several smaller, minor components. These components were not investigated further. Unknown 5 has tentatively been identified as 2-[(isopropylamino)carbonyl]phenylsuIfamic acid and Unknown 6 was identified as 8-hydroxy bentazone. Unknown 4, the major degradation product observed during the study, was proposed to be 3-isopropyl-2,3-dioxo-5-oxocyclopenteno[d]1 H-2,1,3-thiadiazine-4(^{3}H)-one 6 carbonic acid.

The rate of photodegradation of [^{14}C]-Bentazone following continuous irradiation with artificial sunlight was calculated using linear regression analysis assuming first order kinetics. The DT50 value was calculated as 47.8 h; the corresponding DT90 value was 158.8 h.

Comment

Acceptable

STUDY IIA, 7.6/04

Characteristics

reference	:	Philips, M & I.Dawson, (2003)	incubation time	:	See study 7.6/02
study type	:	aqueous photolysis; identification	nominal concentration	:	See study 7.6/02
year of execution	:	2002	pH/temperature	:	See study 7.6/02
GLP statement	:	yes	light intensity	:	See study 7.6/02
guideline	:		Conclusion	:	See below
test substance	:	See study 7.6/02	acceptability	:	**acceptable**
purity	:	See study 7.6/02	Previous evaluation	:	Submitted for renewal (essential)

Study design

The degradation products of study 7.6/02 previously tentatively identified to be 3-isopropyl-2,3-dioxo-5-oxocyclopenteno [d]-1 H-2,1,3-thiadiazine-4(3H)-one-6-carbonic acid, was isolated and purified using solid phase extraction, column chromatography and finally semi-preparative HPLC.
For isolation and Purification of 3-isopropy-2,3-dioxo-5-oxocyclopenteno [d]-1 H-2,1,3-thiadiazine-4(3H)-one-6-carbonic acid (Unknown4) aliquots of test solution were removed after 3, 4, 5, 6 and 7 days irradiation for analysis using the RCP HPLC method. The analysis was conducted to monitor the progress of the reaction. Irradiation was terminated after 7 days and the contents of the quartz vessels combined and stored at ca +4°C. 3-isopropyl-2,3-dioxo-5-oxocyclopenteno[d]-1H-2,1,3-thiadiazine-4(3H)-one-6-carbonicacid was isolated and purified using solid phase extraction, column chromatography and finally semi-preparative HPLC.

Results

Using LC/MS, the mass was confirmed to be 288. LC/MS/MS gave the product ion spectrum derived from the ion with m/z=287.The spectrum was consistent with the proposed structure of the purified photodegradate.

Comment

The study provided gave the confirmation of the identity of Unknown4 as already proposed in study 7.6/02. The other degradation products Unknown1 and Unknown 5 are not further elucidated though in study 7.6/02 it is mentioned that also for Unknown 5 a seperate report is available. This report however, is not submitted. During the peer review a data requirement was set for the identification of all photolysis metabolites occurring >10%. In the study above Unknown 4 was identified as 3-isopropy-2,3-dioxo-5-oxocyclopenteno [d]-1 H-2,1,3-thiadiazine-4(3H)-one-6-carbonic acid and is identical to PkB from study study IIA, 7.6/01. Unknown 5 from the study by Dawson, I., R. Lynn & G.Y. McCorquodale (2003), tentatively identified as 2-[(isopropylamino)carbonyl]phenylsuIfamic acid is identical to PkC from study IIA, 7.6/01.

B.8.4.4 Summary and assessment:

Photo degradation in water

Main notifier

DT_{50} parent: < 5.5 d (tested at pH 5, 7, and 9)

metabolites = Peak B 30%, 17%, 9% at pH 5, 7, 9 respectively

Peak C 21%, 25%, 23% at pH 5, 7, 9 respectively

all others <= 6%

Quantum yield: Fe(III)oxalate actinometer, $4.38 \cdot 10^{-4}$ mol·Einstein^{-1} and 7.7 * 10^{-3} mol/Einstein in aqueous photolysis study (BASF) at pH 5 respectively

Calculated environmental half-lives are 3.6 days (application in March) and 1.6 days (application in May).

Second notifier

DT_{50} parent: 47.8 h

metabolites = unknown 4 (3-isopropyl-2,3-dioxo-5-oxocyclopenteno[d]1 H-2,1,3-

thiadiazine-4(3H)-one 6 carbonic acid); 23.5%

unknown 5 (2-[(isopropylamino)carbonyl]phenylsuIfamic acid); 12.5%

unknown 6 (8-hydroxy Bentazone); 12.6%

unknown 1; 18.2%, 5 minor components

unknown 9; 12%, mixture of minor peaks

Figure 8.4.4-01 Proposed route of degradation of bentazone during aqueous photolysis I BASF scheme

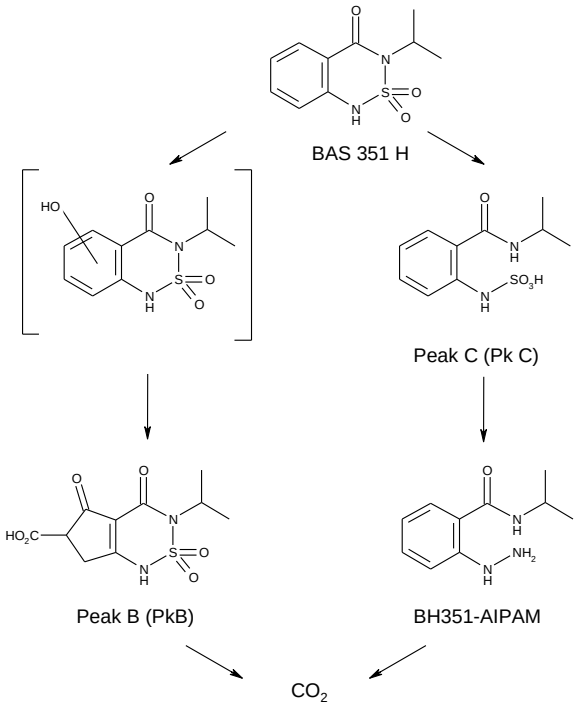
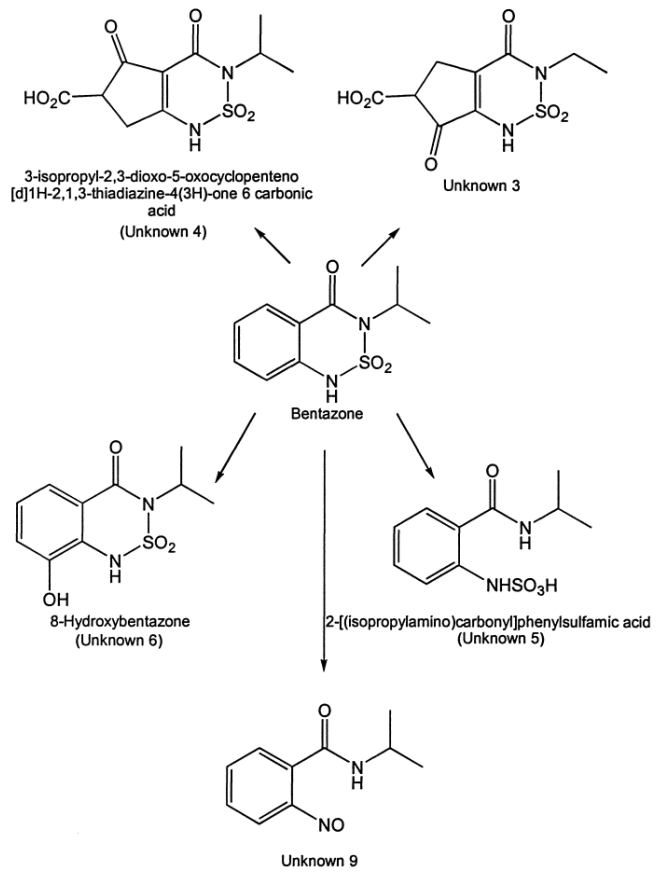


Figure 8.4.4-02 Proposed route of degradation of bentazone during aqueous photolysis II Agrichem scheme



4.1.4.3.2 Soil photolysis

Soil Photolysis

reference	:	Wood, BOD95-00265	GLP statement	:	no
Type of study	:	Soil photolysis	Guideline	:	unknown
Year of execution	:	1986	Acceptability	:	acceptable
test substance	:	^{14}C-U-phenyl bentazone	Previous evaluation	:	In original DAR

Method:

Soil photolysis. The photolysis of ^{14}C-U-phenyl labeled bentazone on a loamy soil at a fortification level of 1.8 kg/ha was studied at 25°C for 941 hours using a water jacketed reaction flask. The light source was a Xenon lamp with a UV filter. Moist air was drawn over the soil during photolysis, and traps were used to capture any volatiles. Dark controls were carried out in the same manner except the vessels were covered with black coated foil.

Soil characteristics:

soiltype	Clay %	Silt %	Sand %	Org.C %	pH	MWHC	CEC [meq/100g]

loam	8.8	46	45.2	0.4	7.2	n.r.	11.9

Results:

^{14}C-Recoveries (in %)

Conditions	light	dark
Min	92.2	98.6
Max	101.4	105.1

Bentazone was completely stable in the absence of light. Results from the photolysis experiments contrast with those from the dark controls in that a small but significant amount of radioactivity is found in the traps. Evidently, the light volatilizes some bentazone or produces volatile transformation products. However, the amount never quite reaches 10 % of the applied radioactivity and seems to cease increasing after about 400 hours of continuous light exposure. Attempts to isolate and characterize the radioactivity in the 1 N sodium hydroxide trap from the 424-hour reaction were frustrated by the low recovery (about 20 %) of radioactivity in ethyl acetate extracts after acidfying the solution. The recovered radioactivity consisted of many bands on TLC.

Comment:

Acceptable.

STUDY IIA, 7.1.3/01

Characteristics

reference	:	Hassink J. 2012(a), Hassink, J. (2012b) amendment	study type	:	soil photolysis
year of execution	:	2012	incubation time	:	up to 15 d
GLP statement	:	yes	nominal concentration	:	7.2 mg/kg
guideline	:	EPA 161-3; EPA 835.2410; SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995); Draft OECD Guideline: Phototransformation of Chemicals in Soil Surfaces (Jan. 2002); EEC 95/36 of 14 July 1995 amending 91/414/EEC	temperature	:	22 ± 1°C
test substance	:	phenyl-U-^{14}C-labelled bentazone	light intensity	:	3 mW/cm^2 (315-400 nm)
purity	:	Chemical purity unlabelled reference 99.8, radiochemical purity 96.5%.	Conclusion	:	study results reliable
soil	:	sandy loam	acceptability	:	**acceptable**

			Previous evaluation	:	Submitted for renewal (essential)

Study design

The test soil (soil properties see Table B.8.1.1.3-01) was collected from the same field (Bruch West) as that used for the aerobic (study 1 of section B.8.1.1.1) soil metabolism studies. A batch of about 800 g of moist sieved (2 mm) soil was adjusted to 40% MWHC and aliquots of 30 g d.w. soil was dispensed into steel dishes (88 mm x 43 mm x 12 mm). Aliquots (216 µL) of phenyl-U-^{14}C-labelled bentazone in acetonitrile were applied to the surface of the soil in each dish (dose 7.2 mg/kg). Treated soil was incubated at 22±1°C for up to 15 days in a SUNTEST unit under continuous irradiation by a Xenon arc lamp (equipped with filter with cut-off at 290 nm) under a continuous humid CO_2 reduced air supply. The light intensity was adjusted to 3 mW/cm^2, which was reported to simulate a clear summer day at Limburgerhof, south Germany (48°N). Volatiles in effluent air were trapped in 0.5M NaOH, 0.5M H_2SO4 and ethylene glycol. In order to maintain the temperature, the inside of the SUNTEST unit was air-conditioned by an external apparatus next to positioning into a water cooling system. The soil temperature during incubation was kept at 22 ± 1°C. Dark control samples were prepared in the same way and incubated in the same apparatus, but the apparatus with the samples were stored in a climatic chamber at 22°C in the dark. Results are presented in table 8.1.1.3-01. Every day all dishes were weighed and water losses were compensated.

Table B.8.1.1.3-01 Properties of study soil

Parameter	**soil Bruch West (10/060/01)**
% Sand/silt/clay(A)	65.9/22.1/11.9
Texture USDA	sandy loam
Texture DIN	loamy sand
pH (water)	7.5
pH (0.01M $CaCl_2$)	7.0
% organic carbon	1.36
CEC (meq/100 g)	9.6
MWHC (g/100 g)	33.4
Microbial biomass	31.5 mg C/100 g

(A) USDA classification system.

Duplicate soil samples were analysed on day 0, 1, 3, 7, 10 and 15 post-treatment. Trapping solutions were sampled and replaced on the same days. Soil samples were extracted three times with methanol and three times with water/methanol (1/1). Radioactivity in extracts and liquid traps was determined by LSC. Soil extracts were pooled and reduce in volume and analysed by LSC and HPLC. Radioactivity in NER was determined by combustion/LSC. NER. Since NER exceeded 10% AR samples underwent extractions with 0.5M NaOH followed by LSC of the extracts and combustion/LSC of the extracted soil (humin). In order to quantify the amount of radioactivity left in the insoluble humines, the soil residue after NaOH extraction was air-dried, homogenized and the weight determined. Three aliquots were combusted and analysed by LSC.

The humic acids were precipitated from the NaOH extracts by adjusting the pH to 1 - 1.5 with conc. HCl (37 %), leaving the fulvic acids in solution. To finish the precipitation completely, the phases were stored over the weekend in a refrigerator and finally centrifuged and decanted into a graduated cylinder. The precipitated residues were dissolved in 0.5 M NaOH. All liquid phases were measured by LSC. The fulvic acid fractions were analysed by HPLC.

– **Results**

The overall mean values for the material balance in the photolysis and the dark control were in the range of 95.3-101.0 % TAR (TableB.8.1.1.3-02 and B.8.1.1.3-03)

Table B.8.1.1.3-02 Distribution of radioactivity in soil Bruch West after treatment with [phenyl-U-14C]-bentazone and incubation under irradiated conditions [%TAR]

DAT	MeOH	MeOH/H_2O	ERR	NER	Volatiles*	Sum
0 DAT I	95.9	4.5	100.4	1.0	n.a.	101.4
0 DAT II	93.6	4.4	98.0	0.6	n.a.	98.6
0 DAT mean	94.7	4.4	99.2	0.8	n.a.	100.0
1 DAT I	84.0	7.9	91.9	7.3	0.5	99.7
1 DAT II	86.7	7.3	94.0	7.8	0.5	102.3
1 DAT mean	85.3	7.6	92.9	7.5	0.5	101.0
3 DAT I	68.3	10.3	78.6	17.3	2.2	98.1
3 DAT II	66.9	11.8	78.7	17.7	2.2	98.6
3 DAT mean	67.6	11.0	78.7	17.5	2.2	98.4
7 DAT I	42.3	15.9	58.2	32.9	5.0	96.1
7 DAT II	39.4	16.2	55.7	33.9	5.0	94.6
7 DAT mean	40.9	16.1	56.9	33.4	5.0	95.4
10 DAT I	45.8	17.9	63.6	28.8	6.5	98.9
10 DAT II	45.2	14.4	59.6	28.5	6.5	94.6
10 DAT mean	45.5	16.1	61.6	28.6	6.5	96.8
15 DAT I	47.2	13.9	61.1	28.7	8.1	97.9
15 DAT II	35.1	14.2	49.3	35.2	8.1	92.6
15 DAT mean	41.2	14.1	55.2	31.9	8.1	95.3

ERR = extractable radioactive residues

NER = non-extractable radioactive residues

* only CO_2 was found

Table B.8.1.1.3-03 Distribution of radioactivity in soil Bruch West after treatment with [phenyl-U-14C]-bentazone and incubation under dark conditions [%TAR]

DAT	MeOH	MeOH/H_2O	ERR	NER	Volatiles*	Sum
0 DAT I	95.9	4.5	100.4	1.0	n.a.	101.4
0 DAT II	93.6	4.4	98.0	0.6	n.a.	98.6

0 DAT mean	94.7	4.4	99.2	0.8	n.a.	100.0
1 DAT I	92.9	3.8	96.7	4.2	0.3	101.2
1 DAT II	91.4	3.7	95.1	4.0	0.3	99.3
1 DAT mean	92.2	3.8	95.9	4.1	0.3	100.3
3 DAT I	91.7	3.0	94.7	6.0	0.6	101.2
3 DAT II	89.9	2.7	92.6	5.7	0.6	99.0
3 DAT mean	90.8	2.8	93.7	5.8	0,6	100.1
7 DAT I	82.1	3.1	85.2	11.7	1.1	97.9
7 DAT II	84.2	3.2	87.4	10.3	1.1	98.8
7 DAT mean	83.2	3.2	86.3	11.0	1.1	98.3
10 DAT I	79.8	3.8	83.6	14.0	1.4	99.0
10 DAT II	81.5	4.1	85.7	13.7	1.4	100.7
10 DAT mean	80.7	4.0	84.7	13.9	1.4	99.9
15 DAT I	71.7	4.3	75.9	20.8	1.8	98.6
15 DAT II	73.0	5.0	78.0	18.4	1.8	98.3
15 DAT mean	72.3	4.6	77.0	19.6	1.8	98.4

ERR = extractable radioactive residues

NER = non-extractable radioactive residues

* only CO_2 was found

Carbon dioxide was the only volatile degradation product trapped. In the sodium hydroxide traps 8.1% TAR were detected after 15 days in the photolysis test and 1.8% TAR after 15 days in the dark control. In H_2SO_4 and ethylene glycol no significant radioactivity could be measured at any time point.

The results of the HPLC analysis are shown in Table B.8.1.1.3-04 and Table B.8.1.1.3-05

Table B.8.1.1.3-04 Radio-HPLC-analysis of soil extracts after treatment of soil Bruch West with [phenyl-U-14C]-bentazone and incubation under irradiated conditions [%TAR]

days after treatment	Bentazone	others*	sum
0 DAT I	99.6	0.8	100.4
0 DAT II	96.8	1.2	98.0
0 DAT mean	98.2	1.0	99.2
1 DAT I	91.2	0.7	91.9
1 DAT II	92.6	1.4	94.0
1 DAT mean	91.9	1.0	92.9
3 DAT I	76.7	1.9	78.6
3 DAT II	76.9	1.8	78.7
3 DAT mean	76.8	1.8	78.7
7 DAT I	52.7	5.5	58.2

days after treatment	Bentazone	others*	sum
7 DAT II	51.9	3.8	55.7
7 DAT mean	52.3	4.6	56.9
10 DAT I	59.9	3.7	63.6
10 DAT II	59.6	0.0	59.6
10 DAT mean	59.7	1.9	61.6
15 DAT I	57.3	3.9	61.1
15 DAT II	40.2	9.1	49.3
15 DAT mean	48.7	6.5	55.2

* each single peak below 4% TAR

Table B.8.1.1.3-05 Radio-HPLC-analysis of soil extracts after treatment of soil Bruch West with [phenyl-U-^{14}C]-bentazone and incubated under dark conditions [%TAR]

days after treatment	**Bentazone**	**others***	**sum**
0 DAT I	99.6	0.8	100.4
0 DAT II	96.8	1.2	98.0
0 DAT mean	98.2	1.0	99.2
1 DAT I	96.7	0.0	96.7
1 DAT II	95.1	0.0	95.1
1 DAT mean	95.9	0.0	95.9
3 DAT I	94.1	0.6	94.7
3 DAT II	92.2	0.4	92.6
3 DAT mean	93.2	0.5	93.7
7 DAT I	85.2	0.0	85.2
7 DAT II	87.4	0.0	87.4
7 DAT mean	86.3	0.0	86.3
10 DAT I	83.6	0.0	83.6
10 DAT II	85.7	0.0	85.7
10 DAT mean	84.7	0.0	84.7
15 DAT I	75.9	0.0	75.9
15 DAT II	78.0	0.0	78.0
15 DAT mean	77.0	0.0	77.0

* each single peak below 1% TAR

Characterization of non-extractable residues

Since the bound residues in soil amounted up to 35% TAR, the already solvent extracted soil was further extracted with NaOH.

The alkali-soluble radioactivity amounted to about 12-24 % in the period of 3 to 15 days after treatment in the soil photolysis. Further fractionation of this alkali-soluble radioactivity showed the major part of the radioactivity could be assigned to the fulvic acid fraction (max. 18.7 % TAR at day 7) which consisted of several unspecific peaks in negligible amounts (Table B.8.1.1.3-06).

In the dark control samples the amount of non-extractable residues was less than under light (Table B.8.1.1.3-06, Table B.8.1.1.3-07). It was confirmed that the alkali-soluble fraction (max. 12.2 % TAR at day 15) consisted of radioactive material mainly assigned to the fulvic acid fraction (max. 6.3 % TAR at day 15).

Table B.8.1.1.3-06 Distribution of radioactivity between fulvic acids and humic acids in soil Bruch West after treatment with 14C-bentazone and incubation under irradiated conditions [%TAR]

DAT	% TAR			
	Sum of NaOH and water extracts	Fulvic acid	Humic acid	Sum*
3 I	11.52	8.52	3.17	11.69
3 II	12.40	9.20	3.37	12.57
3 mean	11.96	8.86	3.27	12.13
7 I	22.46	18.39	4.72	23.11
7 II	23.63	19.09	3.90	22.99
7 mean	23.04	18.74	4.31	23.05
10 I	18.95	15.48	3.20	18.68
10 II	19.78	16.64	2.97	19.61
10 mean	19.37	16.06	3.09	19.15
15 I	23.07	15.30	5.94	21.24
15 II	25.01	16.71	6.26	22.96
15 mean	24.04	16.01	6.10	22.10

* slight deviations from initial values have to be attributed to differing LSC results

Table B.8.1.1.3-07 Distribution of radioactivity between fulvic acids and humic acids in soil Bruch West after treatment with 14C-bentazone and incubation under dark conditions [%TAR]

DAT	% TAR			
	Sum of NaOH and water extracts	Fulvic acid	Humic acid	Sum*
7 I	5.60	4.24	1.41	5.65
7 II	5.29	4.00	1.45	5.45
7 mean	5.44	4.12	1.43	5.55
10 I	6.37	5.04	1.42	6.46
10 II	6.03	4.70	1.51	6.21
10 mean	6.20	4.87	1.46	6.33
15 I	12.48	6.45	4.07	10.52
15 II	11.98	6.14	4.47	10.61

15 mean	12.23	6.30	4.27	10.56

* slight deviations from initial values have to be attributed to differing LSC results

DT_{50} calcuation

Kinetic analysis and calculations of DT50 and DT90 was performed following the recommentdations of the FOCUS kinetics workgroup using the software tool KINGUI. The half-live values (SFO DT_{50}) for bentazone in the test systems were calculated to be 12.8 days under continuous irradiation and 42.1 days in the dark.

Although the FOMC model showed a better fit for both data sets (irradiated soil and the dark control), the fitted kinetic parameters were not significant on a 5 % level. Therefore, the SFO values were regarded as more suitable to describe bentazone degradation in soil.

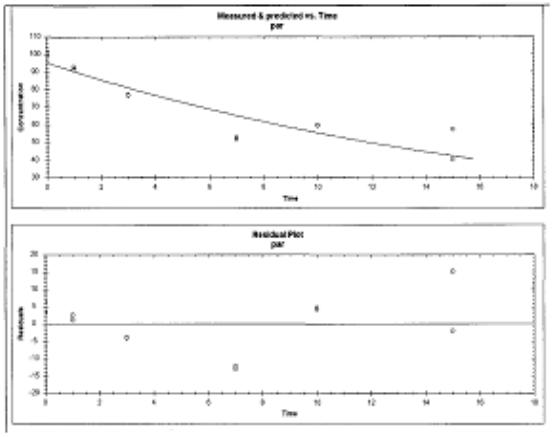
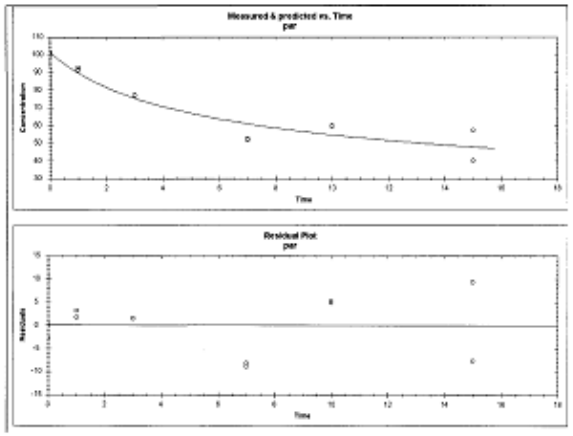
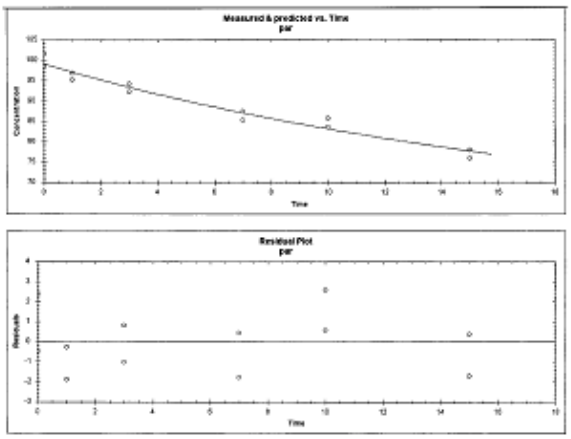
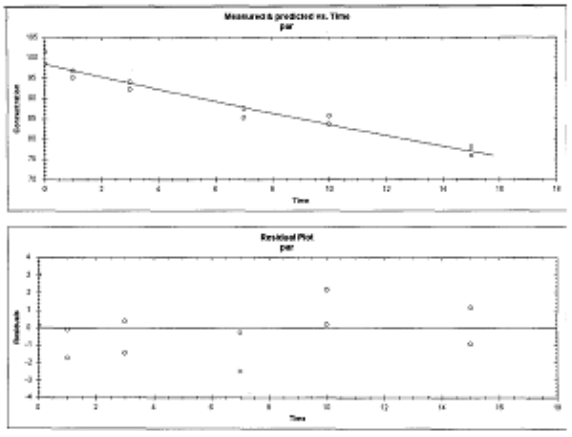
Table B.8.1.1.3-08 Estimated DT_{50} and DT_{90} values of bentazone obtained in soil photolysis study SFO

Kinetic model	Substance	DT_{50} [d]	DT_{90} [d]	χ^2 - error level [%]	Type I error rate (t-prob >0.05)	Visual fit
SFO	soil photolysis	12.8	42.4	7.6	k < 0.05	acceptable
SFO	dark control	42.1	139.7	1.0	k < 0.05	acceptable

Table B.8.1.1.3-09 Estimated DT_{50} and DT_{90} values of bentazone obtained in soil photolysis study FOMC

Sample	DT_{50}	DT_{90}	χ^2 error level	α and β at 5 %level	Visual fit
irradiated	12.6	851	5.0	not significant	acceptable
dark control	76.5	3440	0.9	not significant	acceptable

Fig B.8.1.1.3-01 visual fits of kinetics, irriadiated and dark control SFO (left) and FOMC (right)

Conclusion

The results of the present study showed that sunlight may have an influence on the degradation rate of bentazone in soil. The incorporation into the humic substances was observed to be faster under irradiated than under dark conditions. However, no photodegradates were formed in significant amounts (all peaks < 4%).

Comment

The study is reliable and the results are acceptable.

Second notifier data

STUDY IIA, 7.1.3/02

Characteristics

reference	:	De Vette, H.Q.M, Nachtegaal R.M.A., van Es C. 2002	study type	:	soil photolysis
year of execution	:	2002	incubation time	:	up to 32 d
GLP statement	:	yes	nominal concentration	:	1.92 mg/kg dw soil
guideline	:	SETAC	temperature	:	20 ± 2°C
test substance	:	^{14}C-labelled bentazone position unknown	light intensity	:	Not reported; sunlight at noon, at 55° latitude

purity	:	Chemical purity unlabelled reference 99.8, radiochemical purity 98.4%.	Conclusion	:	study results outdated
soil	:	sandy loam	acceptability	:	**Not acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Study design

Sterilised soils were incubated under aerobic conditions and simulated sunlight. A control was incubated in the dark under the same conditions. The following parameters were determined after sampling times of 0, 4, 7, 14, 21, and 32 days: $^{14}CO_2$ evolution, extractable radioactivity from the solids, and distribution

of radioactivity between parent compound and metabolite(s) by HPLC.

Incubation was carried out under a light source at a distance of approx. 10cm.. The surface of the soil was approximately 2 x 9 cm and the thickness of the soil layer was approximately 0.5 cm.

Evolved $^{14}CO_2$ was trapped in soda lime other volatile were trapped in oil covered glasswool layer. Soil samples were extracted with acetone up to 6 times until the radioactivity was less than 3% of initial. Each extract was subjected to LSC. The extracts were pooled and concentratedand analysed by HPLC. Residual soil residues were determined by a sample oxidizer. The loss of ^{14}C-bentazone (as % of the initial radioactivity) in the soils was fitted to a first order decay equation, using non-linear regression. A first order curve fitting was applied if the $r^2 > 0.7$.

Table B.8.1.1.3-10 Properties of study soil

Parameter	**HW010524**
% Sand/silt/clay[(A)]	58.3/31.13/15.04
Texture	sandy loam
pH (water)	8.7
pH (0.01M $CaCl_2$)	7.6
% organic carbon	1.0
CEC (meq/100 g)	11.4
WHC (g/100 g)	2.3

Results

The distribution of the radioactivity in the irradiated soil and the dark control as percentage of the initial radioactivity is presented in table 8.8.1.1.3-11 and B.8.1.1.3-12, respectively.

Table B.8.1.1.3-11 Distribution of the radioactivity after irradiation with UV-light. Results are expressed as percentage of applied RA.

Time (d)	Volatiles	Radioactive carbon dioxide	Extracts	Bound residue	Recovery
0	n.a.	n.a.	94.6	11.5	106.1
4	0.2	5.7	70.9	18.3	95.2
7	0.1	4.2	62.3	29.2	95.8
14	0.1	13.0	34.5	39.9	87.4
21	0.1	11.0	47.0	27.7	85.8
32	0.5	26.8	32.8	32.9	93.0

n.a. : not applicable

Table B.8.1.1.3-11 Distribution of the radioactivity under dark conditions. Results are expressed as percentage of applied RA.

Time (d)	Volatiles	Radioactive carbon dioxide	Extracts	Bound residue	Recovery
0	n.a.	n.a.	94.6	11.5	106.1
4	0.1	0.6	81.5	16.6	98.7
7	0.0	0.6	91.8	14.9	107.3
14	0.0	0.4	78.9	19.3	98.7
21	0.1	0.9	99.0	11.1	111.1
32	0.1	2.6	63.9	26.9	93.5

n.a. : not applicable

Under UV-light, the amount of radiolabelled carbon dioxide increased to 26.8% of the initial radioactivity after 32 days of incubation. In the dark control the evolved $^{14}CO_2$ increased only to 2.6%. No volatiles were formed in both under both conditions. The extractable radioactivity in the irradiated sandy loam soil decreased from 94.6% of initial activity at the start of the test to 32.8% after 32 days. In the dark control the extractable radioactivity decreased to 63.9% of the initial activity at the end of the test. The bound residue in the irradiated soil increased from 11.5% at the start of the test to a maximum of 39.9% after 14 days. Hereafter the radioactivity decreased The bound residue in the dark control varied between 11.5 and 19.3% and remained almost constant during the test except for day 32, which indicated a decrease in the extracted radioactivity and concomitant increase in bound residue.

The results of the HPLC analyses are reported in table B.8.1.1.3-12.

Table B.8.1.1.3-12 Amounts of [^{14}C] bentazone and metabolites as % A.R. in irradiated soil and dark control.

Time (d)	Irradiated		Dark control	
	Bentazone	Metabolites	Bentazone	Metabolites
0	94.6	n.d.	94.6	n.d.
4	70.9	n.d.	81.5	n.d.
7	62.3	n.d.	91.8	n.d.
14	34.5	n.d.	78.9	n.d.
21	47.0	n.d.	99.0	n.d.
32	32.8	n.d.	63.9	n.d.

n.d. = not detected

Data were fitted to a first order decay using non-linear regression. The resulting DT_{50} and DT_{90} values are reported in table B.8.1.1.3-13.

Table B.8.1.1.3-12 Results of the kinetic analysis for irradiated degradation using firstorder kinetics.

Soil	R^2	A (%)	k_1 (d^{-1})	$t_{½}$ (d) DT50	DT90 (d)
sandy loam soil	0.833	86.5 ± 8.4	0.039 ± 0.010	17.8	59.0

Comment

The study is not according to current guideline. The intensity of the light exposure was not measured. The determination of DT_{50} is not in line with the latest guidance. The study is not considered acceptable. Qualitative information from the study that bentazon is sensible to photolytic degradation to some extend mainly through formation of bound residue, can be used for further assessment as well as the information that no metabolites are formed.

4.2 Environmental fate and other relevant information

4.2.1 Adsorption/desorption

Summary: Adsorption/desorption studies

Soil no	Soil type	cm^3/g	1/n	References
26	loam	37.10	0.991	Redeker, 1978, BOD96-00059 Huber, 1994, BOD95-00267.
27	loamy sand	13.30	1.0275	Redeker, 1978, BOD96-00059 Huber, 1994, BOD95-00267.
28	sand	46.50	1.1251	Redeker, 1978, BOD96-00059 Huber, 1994, BOD95-00267.
29	clay	23.43	0.6642	Keller, 1986, BOD96-00060 Huber, 1994, BOD95-00267.
30	clay	13.22	0.6960	Keller, 1986, BOD96-00060 Huber, 1994, BOD95-00267.
31	clay	175.60	0.6979	Keller, 1986, BOD96-00060 Huber, 1994, BOD95-00267.
32	loamy sand	77.64	0.6882	Keller, 1986, BOD96-00060 Huber, 1994, BOD95-00267.
33	clay sediment rice soil	25.17	0.5614	Keller, 1986, BOD96-00060 Huber, 1994, BOD95-00267.

Summary of data from original DAR.

A brief review of the adsorption/desorption behaviour of bentazone based on the data considered within the former EU review process is provided below.
The EU review report lists K_{oc} values in the range of 13.2 to 175.6 mL g^{-1} (Table B.8.2.1-01, line 1 to 8).

In addition to the studies already peer reviewed and listed in the EU review report of bentazone, three further studies had been included in the adsorption evaluation (Table B.8.2.1-01, line 9 - 12). All existing national registrations of bentazone containing products within the EU since Annex I inclusion in 2000 were based on these 12 data sets.

The sorption of bentazone in soil had to be considered as low as measured in batch equilibrium experiments. The organic carbon related sorption coefficients of bentazone in soil ($K_{f,oc}$ values) ranged from 3.0 to 175.6 mL g^{-1}, with a median $K_{f,oc}$ value of 25.2 mL g^{-1}. The sorption isotherms showed a considerable non-linearity with a median Freundlich exponent of 0.85.

Table B.8.2.1-01 Adsorption values of bentazone in different soils (used since 2000)

No.	Soil Origin	Soil Type	pH	Organic carbon [%]	1/n	$K_{f,oc}$ [mL g^{-1}]	Source	reference
1	Pfungstadt	loam	7.3	0.58	0.99	37.1	EU review report	Redeker (1978)
2	Neuhofen	loamy sand	7.2	2.66	1.03	13.3	EU review report	Redeker (1978)
3	LUFA	sand	7.0	0.51	1.13	46.5	EU review report	Redeker (1978)
4	Monticeller (IL)	clay	5.4	1.80	0.66	23.4	EU review report	Keller (1986)
5	Renvill (MI)	clay	7.7	2.91	0.70	13.2	EU review report	Keller (1986)
6	Briggs (CA)	heavy clay	4.3	1.74	0.70	175.6	EU review report	Keller (1986)
7	Pope Farm (NC)	loamy sand	5.0	0.58	0.69	77.6	EU review report	Keller (1986)
8	Greenville (MS)	clay sediment (rice soil)	6.6	0.70	0.56	25.2	EU review report	Keller (1986)
9	Mellby (Sweden)	sandy loam	6.2	3.40	0.80	49.2	#1994/10464 II A 7.4.1/001	Bergstroem (1994)
10	Vredepeel (NL)	sand	5.2	3.00	0.97	6.4	#1995/10689 II A 7.4.1/002	Keller (1995)
11	Speyrer Wald	sand	6.0	0.70	0.85	3.0	#1999/10685 II A 7.4.1/003	Seher (1999)

12	Borstel	sandy loam	5.7	1.20	0.98	5.9	#1999/10685 II A 7.4.1/003	Seher (1999)
	Median				**0.85**	**25.2**		

Although the adsorption values of the latter three studies had been used for the leaching assessment of bentazone for some years, the studies had never been summarized according to OECD format or peer-reviewed on EU level. For sake of completeness, the summaries of those studies are therefore provided below.

B.8.2.1 Batch sorption

Study IIA, 7.4.1/01

Characteristics

reference	:	Bergstroem L. et al. 1994(b)	soils	:	Sandy loam
year of execution	:	1994	concentrations	:	0.1-5 µg/L
GLP statement	:	No, peer reviewed scientific publication	temperature	:	Not reported
guideline	:	Not clear	$K_F$om	:	49.2 ml/g
test substance	:	BAS 351 H (bentazone)	1/n	:	0.8
purity	:	Not reported	acceptability	:	**Not acceptable**
			Previous evaluation	:	Submitted for renewal (supplementary)

Study design

Bentazone sorption experiments were performed in one Swedish sandy loam soil with a pH of 6.2 and an organic matter content of 5.9 %. The investigation was done within a lysimeter study on four different soil types. Only the results of the sorption experiments are described here.

A sandy loam soil from Mellby, Sweden, was used to determine Freundlich sorption of bentazone.

The characteristics of the soil are summarized in Table B.8.2.1-02.

Table B.8.2.1-02 Characteristics of the experimental soil

Location	**Mellby topsoil**
Depth [cm]	0-23
Soil type (USDA specification)	Sandy loam
%Clay (< 2 µm)	10.4
%Silt (2-50 µm)	10.2

%Sand (50-2000 µm)	79.4
Organic matter [%]	5.9
Organis carbon [%]	3.4
pH(H_2O)	6.2
Porosity [m^3 m^{-3}] layer 0-10 cm Porosity [m^3 m^{-3}] layer 10-20 cm	0.47 0.42
Bulk density [g cm^{-3}] layer 0-10 cm Bulk density [g cm^{-3}] layer 10-20 cm	1.32 1.47

Freundlich sorption of bentazone was measured for topsoil samples (0 - 23 cm) in batch equilibrium experiments with a soil:solution ratio of 1:5.

Bentazone analysis in water samples was performed by hydrolyzing a 200 ml sample with 1 g KOH for 1 h at 100 °C to include esters and bound residues of bentazone. The sample was then acidified with about 5 mL concentrated H_3PO_4 and extracted twice with dichloromethane (50 ± 25 mL), followed by extractive alkylation with pentafluorobenzyl-bromide and evaporation to dryness of the dichloromethane phase. The remainder was re-dissolved in 1.5 mL hexane and analyzed by capillary column gas chromatography, with a detection limit of 0.1 µg/L. Analytical recovery was determined by fortification experiments over a concentration range of 0.1 to 5 µg/L.

Results

Recoveries were between 80% and 120% in fortification experiments over the tested concentration range of 0.1 to 5 µg/L.

The results of the sorption experiment are presented in Table B.8.2.1-. 03

Table B.8.2.1-03 Sorption of bentazone in Mellby topsoil

49.2 toSorption isotherm	**K_d [cm^3 g^{-1}]**	**n [-]**	**r^2 [-]**
Linear	1.67	1.0	0.98
Freundlich	2.90	0.8	0.97

Sorption of bentazone was measured in a sandy loam soil with a pH of 6.2 and an organic matter content of 5.9 %. A Freundlich isotherm was fitted to the data and resulted in a sorption coefficient K_d=2.90 cm^3 g^{-1} and a Freundlich exponent of n=0.8. The corresponding $K_{f,oc}$ is 49.2 mL/g.

Comment:

the study is not considered acceptable. Reviewing the original publication RMS could not find any information on soil solution ratios as included in the applicant summary. The reported analytical method in the above summary is the analytical method for lysimeter leachate and it is not reported if the same method

was applied to the batch sorption. It is not stated how many concentrations were tested in the sorption study and the measurements are not supported by their raw data. The study was not performed according to GLP. No assessment according to the requirements of GLP was provided. The value reported as $K_{f,oc}$ is actually $K_{f,om,}$ the $K_{f,oc}$ would be 85.3 mL/g.

Study IIA, 7.4.1/02

Characteristics

reference	:	Keller W. 1995(b)	soils	:	Loamy sand
year of execution	:	1995	concentrations	:	0.02-25 µg/ml
GLP statement	:	No	temperature	:	20-25 ºC
guideline	:	OECD 106	$K_F$oc	:	6.4 mL/g
test substance	:	bentazon	1/n	:	0.97
purity	:	99.8%	acceptability	:	**Not acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Study design

In laboratory batch experiments the adsorption behaviour of radiolabelled bentazone was investigated on a Vredepeel soil from the Netherlands. The soils had a pH ($CaCl_2$) of 5.2 and an organic carbon content of 3.0 %. It was characterized as a loamy sand. The soil was taken from the top layer 0 - 30 cm. The soil characterization is given in Table B.8.2.1/04

For the determination of the adsorption isotherm, six different concentrations from ca. 0.02 to 25 µg/mL of the test item in 0.01 M $CaCl_2$ solutions were used. The ratio of soil versus test solution was 2/1, and the equilibrium shaking time was 16 hours.

Table B.8.2.1-04 Characterisation of soil used to investigate the adsorption behaviour of bentazone

Soil designation Origin	Vredepeel, 0-30 cm layer Netherlands
Textural class	Loamy sand

Particle size [%]	
>200 µm:	38
20 - 200 µm:	50
6—20 µm:	3
2-6 µm:	1
<2 µm:	8
Organic carbon [%]	3.0
CEC [$cmol^+/kg$]	21.1
pH ($CaCl_2$)	5.2

The soils used were air-dried and sieved to a particle size < 2 mm. The residual water content of the soils was determined by further drying to constant weight at 105 °C. The adsorption study was carried out at room temperature (20 - 25 °C).

To determine the incubation time for reaching equilibrium conditions, experiments were run with a soil / solution ratio of 2/1 (10 g/ 5 mL) for 1, 2, 4, 8, 16 and 24 hours on a mechanical shaker. The ^{14}C-bentazone concentration in the solution was 0.0225 µg/mL. After the corresponding shaking time, the soil-water suspension was centrifuged at 4000 rpm at 20 °C for 15 min. The aqueous phase was decanted and 1 mL aliquots were measured by liquid scintillation counting (LSC). The equilibrium test was carried out in duplicates.

Freundlich adsorption isotherm

Test solutions were prepared in the concentrations as follows by fortifying solution F with increasing amounts of cold bentazone:

Solution	**Bentazone concentration [µg/ml]**
A	25.0225
B	5.0225
C	1.0225
D	0.2225
E	0.0625
F	0.0225

To establish the Freundlich adsorption isotherm, 10 g aliquots of the soil were shaken with 5 mL of the ^{14}C-bentazone test solutions A - F, respectively, for 16 hours on a mechanical shaker. The samples were processed as described above. To show that no test substance adsorbed on the glass wall of the centrifuge tubes, ^{14}C-bentazone test solution F (0.0225 µg/mL ^{14}C-bentazone) without addition of soil was also shaken for 16 hours and analysed by LSC measurement.

Blank soil samples without test substance were also analysed to show that no radioactivity was in the used soil, which could interfere with the ^{14}C-measurements. The Freundlich adsorption isotherm was carried out in triplicates.

Radioactivity in the aqueous supernatants after centrifugation of the soil-water suspension was determined by LSC measurement.

Results

Using six test substance concentrations from ca. 0.02 - ca. 25 µg/mI, a Freundlich adsorption isotherm for bentazone was established. The equilibrium shaking time was 16 hours at room temperature. The soil / water ratio was 2 to 1.

In Vredepeel soil a Freundlich adsorption constant Kf of 0.1933 mL/g and a Freundlich adsorption exponent 1/n of 0.9703 were determined for bentazone. The corresponding Kf,oc value was 6.4 ml/g.

Comment: the study was performed in line with the requirements of OECD 106 and the results are plausible. However, the study was not performed according to GLP. No assessment according to the requirements of GLP was provided. Therefore the study is considered not acceptable according to the current requirements.

Study IIA, 7.4.1/03

Characteristics

reference	:	Seher A. 1999(b)	soils	:	Loamy sand 2x
year of execution	:	1999	concentrations	:	0.04-1 µg/ml
GLP statement	:	Yes	temperature	:	22 ± 2 ºC
guideline	:	OECD 106	$K_F oc$	:	3 – 5.9 mL/g
test substance	:	BAS 351 H	1/n	:	0.849-0.98
purity	:	Purity 99.6%; Radiochemical purity >98%	acceptability	:	**acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Study design

The adsorption behaviour of bentazone was determined on two lysimeter soils. For the determination of the adsorption isotherm, four different concentrations (nominal 5, 1, 0.2 and 0.04 µg/mL) of the test substance in 0.01 M $CaCl_2$ solution were prepared. Two lysimeter soils (both loamy sands), Speyerer Wald and Borstel, were selected for the experiments. The physicochemical soil parameters are listed in Table B.8.2.1/05.

The soil / water ratio was adjusted to 1/1.

Table B.8.2.1-05 Characterisation of soils used to investigate the adsorption behaviour of bentazone

Soil designation **Origin**	**Speyerer Wald** **Germany**	**Borstel** **Germany**
Textural class (German scheme, DIN 4220)	silty sand / loamy sand	sand/ loamy sand/ silty sand
Soil texture [%], (German scheme) Sand Silt Clay	 85 8 7	 85 10 5
Textural class (USDA scheme)	loamy sand	loamy sand
Soil texture [%], (USDA scheme) Sand Silt Clay	 85 7 7	 86 9 5
Organic carbon [%]	0.7	1.2
CEC [$cmol^+/kg$]	5	8
pH ($CaCl_2$) pH (water)	6.0 6.8	5.7 6.3

The soil samples were air-dried and sieved to particle size < 2 mm. The residual water content was determined by drying the soils at 105°C until to weight constant. It ranged between 0.54 and 0.84 % and was taken into account for all calculations.

To determine the time until equilibrium conditions will be reached, samples of 20 g soil and 20 mL solution A containing 1.3 mg ^{14}C-bentazone and 3.7 mg non-labelled bentazone in 1000 mL 0.01 M aqueous $CaCI_2$ solution, were filled in 150 mL centrifuge glass tubes. The tubes were covered with parafilm to avoid evaporation of water. The tubes were gently shaken on a mechanical shaker for 1, 2, 4, 8, 16 and 24 hours in the dark. The soil/water suspension was then centrifuged and the supernatant was decanted from the soil residue. An aliquot of the supernatant was then radio-assayed. A control was carried out with 20 g soil and 20 mL 0.01 M aqueous $CaCl_2$ solution without test substance. The results showed that the equilibrium time was reached after 4 hours.

To check if the test substance was adsorbed to centrifuge glass tube, a 20 mL aliquot of the solution A was also carried out without soil by shaking 24 hours along with the equilibrium test.

To determine the adsorption isotherm the four test substance concentrations of nominal 5, 1, 0.2 and 0.04 µg/mL) were prepared and the adsorption isotherm determination test was performed with each soil as

described for the adsorption equilibrium test. After centrifugation and decantation, the volume of the supernatant was measured by weighing and an aliquot analysed by LSC.

Results

The Freundlich adsorption coefficients K_f and the corresponding Freundlich adsorption exponent 1/n as well as the corresponding $K_{f,oc}$ values are given in Table B.8.2.1/06. The Freundlich adsorption exponent 1/n indicated a non-linearity of the adsorption with the concentration.

Table B.8.2.1/06 Results from the adsorption experiments with bentazone

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
Speyerer Wald (98/1287)	Loamy sand	0.7	6.0	0.021	0.849	3.0
Borstel (99/1332)	Loamy sand	1.2	5.7	0.071	0.980	5.9

A mass balance for the two soils was not established. However the stability of the test item was proven by HPLC in the aqueous solution after adsorption for the test solution. The purity of the test item was 99.58% in soil Speyerer Wald and 99.61% in soil Borstel indicating stability of the test item in the solution.

Comments

The study was performed in line with the requirements of OECD 106 and the results are plausible and acceptable.

Study IIA, 7.4.1/04; 7.4.1/05; 7.4.1/06

Characteristics

reference	:	Boivin A. 2003(b); thesis Boivin A. et al. 2005(b); publication Boivin A. et al. 2005(c) ; publication	soils	:	
year of execution	:	2003 resp 2005	concentrations	:	0.25-10 mg/L
GLP statement	:	No	temperature	:	
guideline	:	OECD 106	$K_F$oc	:	
test substance	:	[U-^{14}C-phenyl]bentazone	1/n	:	
purity	:	>99% radiochemical purity	acceptability	:	**acceptable**
			Previous		Submitted for

			evaluation	:	renewal (essential)

Study design

In laboratory batch experiments the adsorption / desorption behaviour of five pesticides including bentazone was investigated on thirteen European agricultural top soils according to the current version of the OECD guidance 106 (2000). The soils covered a range of pH (H_2O) from 5.3 to 8.2, a range of organic matter content from 1.08 to 6.03% and different textural classes. In the following the data obtained for bentazone are reviewed.

For the determination of the adsorption isotherm, four different concentrations (nominal 0.25, 1, 5 and 10 mg/l) of the test item in 0.01 M $CaCl_2$ solutions were used. Non-radiolabelled and ^{14}C-labelled bentazone was mixed to obtain the respective concentrations. The ratio of soil versus test solution was 1/5, and the measurements were performed at the adsorption equilibrium time of 16 hours for the thirteen soils. Desorption was also investigated starting at a concentration of 1 mg/L. Supernatants were replaced by aqueous $CaCl_2$ solution and tubes were shaken for 16 h at 20±2 °C. The desorption process was repeated until the supernatant radioactivity became less than three times the liquid scintillation analyser background noise.

Thirteen cultivated soils were sampled in the surface layers (0 - 15 cm). They were selected on the basis of their texture, organic matter contents and pH values. Twelve soils came from Lorraine area (France) and one from Brittany (Experimental station of Kerlavic; France). Soil types were classified into cambis ols, calcisols and regosols according to the FAO classification [cited from Boivin *et al.* 2005].

The physico-chemical characterization of the soils is provided in Table B.8.2.1/07.

Table B.8.2.1-07 Characterization of soils used to investigate the adsorption behaviour of bentazone

Soil type	**Clay [%]**	**Loam [%]**	**Sand [%]**	**Organic matter content [%]$^{\$}$**	**pH_{H2O} [-]**	**Organic carbon-nitrogen ratio [-]**	**Cation exchange capacity [cmol/kg]**
Dystic Cambisol	17.7	45.2	37.1	6.03 (3.47)	5.3	10.50	13.5
Stagnic Cambisol	53.6	39.7	6.7	5.81 (3.42)	6.7	9	31.5
Calcaric Regosol 2	38.1	34.2	27.7	5.59 (3.26)	8.2	8.83	24
Fluvic Gleyic Cambisol	51.6	42.9	5.5	4.80 (2.78)	6.2	8.66	25.4
Eutric Cambisol 2	10.4	19	70.6	3.66 (2.10)	6.2	10.47	7.9
Calcaric Regosol 1	33.4	25	41.6	3.63 (2.08)	7.9	8.66	14.7
Cambic Calcisol	45.5	44.3	10.2	3.64 (2.12)	8.1	10.59	16.9

Soil type	Clay [%]	Loam [%]	Sand [%]	Organic matter content [%][$]	pH_{H2O} [-]	Organic carbon-nitrogen ratio [-]	Cation exchange capacity [cmol/kg]
Vertic Stagnic Cambisol	41.1	48.6	10.3	3.32 (1.98)	6.9	9.90	18.7
Cambic Stagnic Vertic Calcisol	50.9	34.8	14.3	2.77 (1.58)	8	7.92	21.2
Stagnic Luvisol	30.9	50	19.1	2.62 (1.55)	5.9	9.13	5.5
Fluvic Stagnic Cambisol	11.4	22.5	66.1	1.50 (0.86)	5.8	9.6	14.8
Eutric Cambisol 1	12.7	13.8	73.5	1.43 (0.81)	6.4	10.13	4.6
Fluvic Cambisol	20.1	55.4	24.5	1.08 (0.63)	5.5	9.29	9.6

[$] organic carbon content in brackets

Soil samples were air-dried, sieved to 2-mm, stored in the dark at room temperature (20 ± 2 °C) and sheltered from humidity.

Radioactivity in the aqueous supernatants was determined by liquid scintillation counting (LSC). Several tubes without soil were also shaken to serve as a control; they showed no loss of ^{14}C. Thus, differences between the initial and equilibrium concentrations were assumed to be due to sorption onto soil.

Results

The Freundlich adsorption coefficients K_f ranged from 1.2 to 1.9 mL/g for the thirteen soils (Table B.8.2.1 0/8). Ranging from 1/n = 0.99 to 1, the Freundlich adsorption exponent indicated linearity of the adsorption with the concentration. On average, 22 ± 2.3 % of the total amount were adsorbed.

Table B.8.2.1-08 Results from the adsorption experiments with bentazone

Soil type	pH_{H2O} [-]	Adsorption [%]	K_f [mL/g]	$K_{f,oc}^*$ [mL/g]	1/n [-]
Dystic Cambisol	5.3	28 ± 1	1.9 ± 0.2	55	0.99
Stagnic Cambisol	6.7	22 ± 1	1.4 ± 0.1	41	1
Calcaric Regosol 2	8.2	20 ± 2	1.2 ± 0.1	36	0.99
Fluvic Gleyic Cambisol	6.2	23 ± 1	1.5 ± 0.1	54	1
Eutric Cambisol 2	6.2	21 ± 1	1.3 ± 0.2	60	1
Calcaric Regosol 1	7.9	20 ± 2	1.2 ± 0.1	63	1
Cambic Calcisol	8.1	20 ± 4	1.3 ± 0.1	55	1

Soil type	**pH_{H2O} [-]**	**Adsorption [%]**	**K_f [mL/g]**	**$K_{f,oc}$* [mL/g]**	**1/n [-]**
Vertic Stagnic Cambisol	6.9	20 ± 1	1.2 ± 0.1	63	1
Cambic Stagnic Vertic Calcisol	8	20 ± 1	1.3 ± 0.1	79	1
Stagnic Luvisol	5.9	22 ± 1	1.4 ± 0.1	93	1
Fluvic Stagnic Cambisol	5.8	22 ± 1	1.4 ± 0.1	137	1
Eutric Cambisol 1	6.4	20 ± 1	1.2 ± 0.1	158	0.99
Fluvic Cambisol	5.5	23 ± 2	1.5 ± 0.1	144	0.99

* Taken from II A 7.4.1/4 – Boivin A. (2003b) (page 64)

Principal component analysis corroborated this finding, indicating that K_f values and soil pH were inversely related, while other soil components showed no or little effect. The two main principal component axes could explain 94.57% of the total variability.

Furthermore, multiple linear regression was used to relate K_f values with organic matter content and soil pH. Freundlich adsorption coefficients K_f ranged from 1.2 to 1.9 mL/g for the thirteen soils. Freundlich exponents were between 0.99 and 1.0 indicating linearity of the adsorption isotherm. $K_{f,oc}$ –values range from 36 to 158 mL/g. A correlation was found between soil pH and K_f value, with slightly higher adsorption at lower soil pH. Using multiple linear regression a model was found where bentazone K_f values were predicted using soil organic matter content and pH.

Desorption behaviour

A large part of bentazone could be desorbed from the soil without any significant differences between the thirteen soils (96.9% ± 4.1%). No significant multiple correlation coefficient could be found for the relationship between desorption and the soil properties organic matter and pH.

Comment:

the study was performed in line with the requirements of OECD 106 and the results are plausible. De soils are according to the requirements of OECD 106 a differ sufficiently in their properties. However, the study was not performed according to GLP. No assessment according to the requirements of GLP was provided. As the study is a peer reviewed scientific publication the results from the publications Boivinet al. 2005 (b and c) are therefore considered acceptable information. The study Boivin A. 2003(b) is in French contains however a large number of raw data. Therefore it is used to verify all data.

Study IIA, 7.4.1/07

Characteristics

reference	:	Boivin A. 2004(b); publication	soils	:	
year of execution	:	2004	concentrations	:	0.5 mg/50 g
GLP statement	:	No	temperature	:	20ºC
guideline	:	none	$K_F$oc	:	n.r.
test substance	:	[U-^{14}C-phenyl]bentazone	1/n	:	n.r.
purity	:	>99% radiochemical purity	acceptability	:	**Not acceptable**
			Previous evaluation	:	Submitted for renewal (supplementary)

Study design

The sorption and degradation behaviour of bentazone was investigated in three French soils over a time range of 160 days. The soils were characterized as a clay soil, a loamy soil and a sandy soil. A summary of the soil characteristics is given in Table B.8.2.1-09

Table B.8.2.1-09 Properties of soils used to investigate sorption and degradation of bentazone under aerobic conditions

	Clay (%)	**Loam (%)**	**Sand (%)**	**Organic matter content (%)**	**pH_{H2O}**	**Linked water [a] (cm^3 cm^{-3})**	**Mobile water [b] (cm^3 cm^{-3})**	**Organic carbon/ nitrogen ratio**	**Cation exchange capacity (cmol kg^{-1})**
Vertic stagnic cambisol (clay soil)	41	49	10	3.3	7	0.39	0.13	9.9	18.7
Stagnic luvisol (loamy soil)	31	50	19	2.5	5.9	0.33	0.10	9.1	14.8
Fluvic stagnic cambisol (sandy soil)	11	23	66	1.5	5.8	0.27	0.21	9.6	5.5

a Soil water content estimated at -20 mm H_2O.

b Soil water content estimated at saturation (θ_S).

The soils (0-15 cm layer) were passed through a 2 mm sieve before use. For treatment, a mixture of radiolabeled (phenyl-U-^{14}C) and non-radiolabeled bentazone was used. The test item was applied in separate batch applications at a nominal rate of 0.5 mg bentazone per sample (50 g soil) which, according to the authors, corresponds to a field application rate of 1200 g bentazone per hectare.
Soil batches were incubated in the dark under aerobic conditions at a soil moisture of 80% field capacity and a temperature of 20°C. Each soil sample was placed in a glass dish in an individual airtight jar (1.5L), containing flasks with aqueous sodium hydroxide (10 ml 0.5 M) for trapping evolving $^{14}CO_2$, and with distilled water for keeping sufficient humidity in the surrounding atmosphere to reduce water losses from the soil. Samples were taken at 0, 7, 15, 30, 60 and 160 days after treatment (DAT).
Soil samples were extracted several times with 0.01 M $CaCl_2$ solution for 16 h, respectively, until the supernatant radioactivity became less than three times the background radioactivity. Subsequently, the soil samples were extracted with methanol until the supernatant radioactivity became less than three times the background radioactivity. The individual extracts were analysed by liquid scintillation counting (LSC). The remaining soil after extraction was combusted in order to determine the amount of non-extractable soil bound residues. To establish extraction kinetics for the extraction with 0.01 M $CaCl_2$ solution, subsamples of the extraction supernatant were taken after 1, 2, 5, 10, 20, 30, 60, 180, 420 and 1200 min of shaking. Approximately 90% of applied bentazone was released in less than 1 h and a quasi-equilibrium state was reached after 2 h.

Results

At the beginning of the investigations, the amount of $CaCl_2$-extractable bentazone was high (92 – 97% of applied radioactivity at day 0) but decreased over time to 11-51% of applied radioactivity after 160 days of incubation. The number of extractions necessary to release the water-extractable portion of bentazone increased at increasing incubation time. No information was given on the amount of bentazone in the $CaCl_2$ and the following methanol extracts. Therefore, a clear time dependent sorption of bentazone in soil could not be calculated. The reduced extractability of bentazone with $CaCl_2$ solution over time described in this publication is thus due to degradation processes which led to tight binding and incorporation into the organic soil matrix.

Comment

The publication tried to give insight in time dependent sorption behaviour of bentazone. The study showed some defecies already mentioned in the summary and evaluation. Furthermore the study is pre current developments regarding aged sorption. The current results are not usefull to use in further assessment.

Study IIA, 7.4.1/08

Characteristics

reference	:	Larsbo et al 2009(a);	soils	:	

		publication			
year of execution	:	2009	concentrations	:	0.5 mg/50 g
GLP statement	:	No	temperature	:	20ºC
guideline	:	OECD 106	K_F	:	0.148 to 0.567 mL/g,
test substance	:	[U-^{14}C-phenyl]bentazone unlabelled bentazone	1/n	:	0.762-1.11
purity	:	100% radiochemical purity resp 97% chemical purity	acceptability	:	**acceptable**
			Previous evaluation	:	Submitted for renewal (supplementary)

Study design

In laboratory experiments, the adsorption behaviour of bentazone was determined on three Swedish soils under different tillage systems according with the OECD guideline 106 (2000). For all sites conventional tillage (CT) means shallow cultivation (stubble cultivation by discing or chiseling) followed by moldboard ploughing. Reduced tillage (RT) means only shallow cultivation. The depth of influence for CT and RT is about 25 and 10 cm, respectively. The physico-chemical characterization of the soils is provided in Table B.8.2.1/10.

Table B.8.2.1-10 Characterization of soils used to investigate the adsorption behaviour of bentazone

Soil designation	**Ultuna 59°49'N; 17°39'E**			**Säby 59°50'N; 17°42'E**			**Lönnstorp 55°40'N; 13°6'E**		
Textural class	Silty clay			loam			Sandy loam		
Depth [cm]	0-20	0-5	10-20 **	0-20	0-5	10-20 **	0-20	0-5	10-20 **
Tillage *	CT	RT	RT	CT	RT	RT	CT	RT	RT
Clay (<2 µm)	45.8	44.0	51.7	21.0	21.3	19.5	16.0	15.6	15.5
Silt (2–60 µm)	39.2	42.4	40.9	47.3	50.8	48.2	27.2	27.8	28.0
Sand (60-200 µm)	15.0	13.6	7.4	31.7	27.9	32.3	56.8	56.6	56.5
pH (H_2O) **	5.34 ±0.42	5.63 ±0.18	5.67 ±0.036	5.56 ±0.19	5.71 ±0.11	5.71 ±0.15	6.73 ±0.53	6.22 ±0.07	6.37 ±0.035
Total organic carbon [%] **	1.97 ±0.45	2.57 ±0.18	1.94 ±0.45	2.75 ±0.1	3.04 ±0.007	2.45 ±0.14	1.8 ±0.23	2.14 ±0.034	1.79 ±0.14

* CT = conventional tillage, RT = reduced tillage

** The total organic carbon content and pH were measured in samples from 12–17 cm depth.

Results are given ± standard deviation (n=4 for Ultuna and Säby; n=3 for Lönnstorp)

Adsorption data were obtained for five different concentrations in the range of 0.075–10 µg/g dry weight for bentazone for two replicate samples between 0–5 and 10–20 cm depths for RT ($RT_{0–5\ cm}$, $RT_{10–20\ cm}$) and the 0–20 cm depth for CT for all sites. After shaking for 24 hours, the tubes were centrifuged after which the radioactivity was measured in 1 mL of the supernatant by liquid scintillation counting. Tubes without soil and ^{14}C-labeled substances were included for subtraction of background radiation, and tubes without soil were used to give the initial amount of ^{14}C activity added. Bentazone was extracted from the soil samples with 20 mL of methanol by shaking for 1 h at 200 rpm. The samples were centrifuged and 1 mL aliquots of the supernatants were analysed using high performance liquid chromatography (HPLC).

Results

The recoveries for bentazone were 114–119% for all the studied soils. The limit of quantification (LOQ) was 0.07 mg/g dry weight. The limit of detection (LOD) was determined to be one third of this value.

No significant adsorption of bentazone occurred on the glass tubes.

The Freundlich isotherm was fitted to the measured data using linear regression on log-transformed data. Freundlich adsorption coefficients K_f covered a range from 0.148 to 0.567 mL/g, with the Freundlich adsorption exponent ranging from 1/n = 0.767 to 1.11. The results are summarized in Table B.8.2.1/11. Freundlich adsorption coefficients were largest (though not always significantly) for $RT_{0–5\ cm}$ for all sites. Since the differences in n_f-values were in most cases small, this indicates stronger sorption for $RT_{0–5\ cm}$ at all concentrations.

Table B.8.2.1-11 Results from the adsorption experiments with bentazone in Swedish soils under different tillage systems

Soil			**K_f [mL/g]**	**1/n [-]**
Ultuna	CT *	0–20 cm	0.479	0.846
Ultuna	RT *	0–5 cm	0.567	0.777
Ultuna	RT	10–20 cm	0.557	0.767
Säby	CT	0–20 cm	0.371	0.847
Säby	RT	0–5 cm	0.396	0.864
Säby	RT	10–20 cm	0.339	0.857
Lönnstorp	CT	0–20 cm	0.323	1.11
Lönnstorp	RT	0–5 c m	0.440	0.942

Lönnstorp RT 10–20 cm	0.148	0.880

* CT = conventional tillage, RT = reduced tillage

Comment

The results of the study are from a scientific publication. The results are well reported and parts of the study is performed in line with guideline OECD 106. The study is not subjected to GLP but is a peer reviewed publication. However, the aim of the study was to investigate the effect of agricultural practices (tillage) on the sorption and related leaching behaviour. Different tillage systems and different depths are tested per soil type. It is unclear if these should be considered replicates or not. The results are considered additional information by RMS. During the peer review it was discussed that the results for each soil layer should be considered as replicates and the arithmetic mean value of those for each soil is acceptable for risk assessment.

Second notifier data

Study IIA, 7.4.1/09

Characteristics

reference	:	Völkel, W	soils	:	Loamy sand
year of execution	:	2001	concentrations	:	4.87 mg/l.
GLP statement	:	Yes	temperature	:	20ºC
guideline	:	OECD 106	$K_F$oc	:	15 cm^3/g
test substance	:	benzenering-U-14C]-Bentazone	1/n	:	-
purity	:	>98% radiochemical purity; chemical purity 99.9%	acceptability	:	**Not acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Study design

In a preliminary test the adsorption behaviour of ^{14}C-bentazone was tested using three different soil dry weight to aqueous phase ratios. After 2, 4, 24 and 48 hours of agitation, aliquots of the aqueous phase were analysed by LSC. Equilibrium was reached after about two to four hours. The control samples showed no adsorption of the test item on the tube walls. The mass balance was carried out for all samples. After adsorption, the aqueous phase of each tube was decanted and the soil was extracted three times using acetonitrile/water (8:2; (v/v)), in order to show the extractability of the test item. The radioactivity remaining in soil was determined by combustion of soil aliquots (in triplicate, about 1 g each). The amount of pore water remaining in the soil was calculated and the radioactivity in that water was reduced from the

combustion result. The extracts resulting from the same tube were combined and quantified by LSC. The aqueous supernatant and the combined extracts of each tube were submitted to HPLC analysis.

Table B.8.2.1-12 Characteristics of the experimental soil

Location	**Borsteltopsoil**
Depth [cm]	0-30
Soil type (USDA specification)	loamysand
%Clay (< 2 µm) %Silt (2-50 µm) %Sand (50-2000 µm)	6.47 12.65 80.88
Organic matter [%]	3.05
Organis carbon [%]	1.77
pH(KCl)	5.6
CEC (mmol/kg soil)	7.12

Results

In the preliminary test the equilibration time for the adsorption was determined and it was seen that equilibration was reached after about two to four hours of adsorption. Koc values were calculated for all soil to aqueous phase ratios. Based on the amount of substance in the supernatant, the 1/1 ratio was appropriate, showing 4% adsorption of the
amount applied. The resulting Koc value was 15 cm^3/g.

Comment

Only a preliminary test was done and in the study the soil solution ratio was varied to find the optimum.The resulting Koc value based on the Kd at soil solution ratios are included. NOT reported the result at a soil solution ration of 1/1 as the determined Koc. RMS is of the opinion it is not appropriate to use this value for further assessment.

Study IIA, 7.4.1/10

Characteristics

reference	:	Vonk J.W. & van den Hoven A.W.	soils	:	Dich sediment 2 types
year of execution	:	1987	concentrations	:	0.5-4 mg/10ml.
GLP statement	:	no	temperature	:	20ºC
guideline	:	Not reported	$K_F$oc	:	

test substance	:	Bentazone	1/n	:	-
purity	:	chemical purity >99%	acceptability	:	**Not acceptable**
			Previous evaluation	:	Submitted for renewal (supplementary)

Study design

1.0 g portions of.sediment (dry weight; this is 1.58 g of wet sediment 1 and 5.75 g of wet sediment 2) were placed in 25 ml screw-cap flasks. To these sediment portions 4.00, 2.00, 1.00 and 0.50 mg of bentazone in 0.01 M CaSO, solution was added and the liquid phase was made up with CaSO, solution to a final volume of 10 ml. After shaking for 24 hours at 20°C the flasks were centrifuged. Bentazone was determined in samples of the clear supernatant. Analysis was carried out by HPLC on a reverse phase column. Quantitative determination was performed by UV detection with external calibration.

Table B.8.2.1-13 Characteristics of the experimental sediments

Location	**Sediment 1**	**Sediment 2**
%Clay (< 2 µm)	12.2	34.1
%Silt (2-50 µm)	11.1	12.5
%Sand (50-2000 µm)	81.5	19.0
Organic matter [%]	2.1	29.8
$CaCO_3$ [%]	4.2	17.1
pH(KCl)	7.5	7.0

Results

Table B.8.2.1-14 Distribution of bentazone between solution and two types of ditchbottom sediment.

Amount of bentazone (µg)	Sediment 1		Sediment 2	
	In solution (µg/ml)	Adsorbed (µg/g)	In solution (µg/ml)	Adsorbed (µg/g)
4000	390 (410)	100 (NA)*	410 (420)	NA (NA)
2000	190 (200)	100 (NA)	170 (46)**	300 (-)
1000	96 (96)	40 (40)	97 (98)	30 (20)
500	50 (20)**	NA (-)	52 (51)	NA (NA)

( ) = duplicate value
* = no adsorption
** = turbid supernatant

The adsorption of bentazone to the ditch-bottom sediments is so weak or absent that from the data of Table no reliable isotherms can be plotted.

Comment

The study was performed with ditch bottom sediments. From the results no reliable sorption isotherms for sediment can be plotted. The study is not relevant for soil adsorption of bentazone.

Metabolites

Main notifier data

Study IIA, 7.4.2/01

Characteristics

reference	:	Tornisielo A., Vasques A.C. 2011(b)	soils	:	2X loamy sand, 1X sand
year of execution	:		concentrations	:	0.05-5 µg/mL
GLP statement	:	yes	temperature	:	20°C
guideline	:	OECD 106	K_F	:	1.62-2.79
test substance	:	BH 351-N-Me	1/n	:	0.80-0.92
purity	:	Chemical purity 99.6%	acceptability	:	**acceptable**
			Previous evaluation	:	Submitted for renewal (essential)

Study design

In laboratory experiments the adsorption behaviour of the bentazone metabolite N-methyl-bentazone (Reg. No. 79520) was investigated on three European soils. The three soils covered a range of pH (in $CaCl_2$) from

5.2 to 7.5, a range of organic carbon content from 0.52 % to 1.15 % and two different DIN textural classes: loamy sand and sand. The physico-chemical characterisation of the soils is provided in Table B.8.2.1/15. The soils used were sieved to a particle size <2 mm and air-dried at ambient temperature. The actual water content of the soils was determined. The residual water content for the adsorption test was between 0.31 % and 0.94 %, which was taken into account for all calculations.
For the determination of the adsorption isotherm, five different concentrations (nominal 5.0, 2.5, 1.0, 0.5 and 0.05 µg/mL) of the test item in 0.01 M $CaCl_2$ solutions were used. The ratio of soil to test solution was 1/2, and the measurements were performed at the adsorption equilibrium time of 24 hours for all three soils.

Table B.8.2.1-15 Characterisation of soils used to investigate the adsorption behaviour of N-methyl-bentazone

Soil designation Origin	LUFA 5M	LUFA 2.3	LUFA 2.1
Soil texture [%] (German scheme) % Sand % Silt % Clay	 52.1 34.7 13.2	 54.8 34.0 11.2	 88.2 8.9 2.9
Textural class (USDA)	Sandy loam	Sandy loam	Sand
Soil texture [%] (USDA) % Sand % Silt % Clay	 56.0 30.7 13.2	 56.8 32.0 11.2	 89.1 8.0 2.9
Organic carbon [%]	1.15	1.09	0.52
CEC [$cmol^+/kg$]	13.1	10.4	2.0
pH (water)	8.4	7.9	6.3
pH ($CaCl_2$)	7.5	6.9	5.2

A preliminary experiment was run with two soils to find the optimal soil/solution ratio for the adsorption/desorption tests and to determine the incubation time needed to reach equilibrium conditions. A soil/solution ratio of 1/2 and an equilibrium time of 24 h were found to be appropriate.
Adsorption to the container material was also investigated as well as stability of N-methyl-bentazone in solution. Fortification experiments in solution were performed both on $CaCl_2$ supernatant from control samples and on control soils. Aliquots of 0.95 mL of $CaCl_2$ supernatant were fortified with 0.05 mL of

solutions at concentrations of 0.100 µg/mL and 100 µg/mL, resulting in concentrations of 0.005 µg/ml and 5.0 µg/mL, respectively.
In addition, untreated soil samples were fortified with N-methyl-bentazone. Control soil samples of 5 g were fortified with 0.05 mL of solutions in the concentrations of 1.0 µg/mL and 1000 µg/mL, resulting in concentrations of 0.01 mg/kg and 18.0 mg/kg. The soil samples were then extracted and centrifuged the same way as the samples of the adsorption experiments. All fortification samples were determined in triplicate by HPLC-MS/MS.
For the Freundlich adsorption isotherm determinations, the test soil were shaken for 24 hours with 10 mL of test solution, in glass tubes. Tubes were centrifuged and the supernatant was collected for analysis, taking into account the weight of the soil and the remaining solution.
The remaining soil in the tubes was extracted with 50 mL of methanol/water 1/1 (v/v) solution. The supernatants were then filtered with 0.2 µm teflon filters.
Analysis of initial solutions, supernatants and soil extracts, diluted to appropriate extent with methanol/water (1/1, v/v) was performed by LC-MS/MS.
The limit of quantitation (LOQ) for the $CaCl_2$ supernatant was 0.005 µg/ml, corresponding to 10 % of the lowest nominal test concentration.

The LOQ for the soil extracts was 0.01 mg/kg dry soil, using a previously validated method (method L0136/01).

Results

The Freundlich adsorption coefficients K_f covered a range from 1.620 (LUFA 2.1 soil) to 2.791 mL/g (LUFA 2.3 soil) for the three soils. The $K_{f,oc}$ values ranged from 204.7 mL/g (LUFA 5M soil) to 311.6 mL/g (LUFA 2.1 soil), with the Freundlich adsorption exponent ranging from 1/n = 0.80 (LUFA 2.3 soil) to 0.92 (LUFA 2.1 soil). The results are summarised in Table B.8.2.1-16.

Table B.8.2.1-16 Results from the adsorption experiments with N-methyl-bentazone

Soil	**Soil Type (DIN)**	**Org. C [%]**	**pH ($CaCl_2$)**	**K_f [mL/g]**	**1/n [-]**	**$K_{f,oc}$ [mL/g]**
LUFA 5M	Loamy Sand	1.15	7.5	2.354	0.88	204.7
LUFA 2.3	Loamy Sand	1.09	6.9	2.791	0.80	256.1
LUFA 2.1	Sand	0.52	5.2	1.620	0.92	311.6

A mass balance determination was carried out for all soils and all concentrations from the Freundlich adsorption isotherm determination. For test item N-methyl-bentazone, the values for the recovery ranged from 96.2 % to 106.4 % for LUFA 5M soil, 81.6 % to 105.1 % for LUFA 2.3 soil and 96.5 % to 107.4 % for LUFA 2.1 soil.

Comment

The study is performed according to current guideline. The results are acceptable and can be used for risk assessment.

Second notifier data

The second notifier did not submit any studies regarding the sorption of metabolite N-methyl bentazone. In the summary document MII a statement is provided.

No information is available concerning the soil adsorption of N-methyl bentazone, however, from a physicochemical viewpoint, little difference is expected between the soil adsorption of parent molecule bentazone, and metabolite N-methyl bentazone – if anything, the adsorption of N-methyl bentazone, being slightly more hydrophobic, to soil will be higher than that of the parent compound. As such the leaching assessment for N-methyl bentazone will initially be done by setting K_{OM} equal to the K_{OM} for bentazone – this will represent a worst case situation. If necessary, an adsorption/desorption study can be done; in such a case, there is no need to do this as a full adsorption/desorption study – a tier 2 study, determining KD at a single concentration would suffice to refine PEC_{GW} calculations, if necessary.

Comment

RMS considers the statement not acceptable.

4.2.2 Votalisation

B.8.7. Fate and behaviour in air (Annex IIA 7.2.2; Annex IIIA 9.3) New OECD number 7.10

Main notifier

Based on its physical-chemical properties, bentazone has no potential for volatilisation (4.9×10^{-6} Pa at 20°C). Furthermore, volatilisation from moist soil surfaces is not expected to be an important process for bentazone based on a Henry's law constant of 2.108 10^{-9} kPa m³/mol. Consequently bentazone is considered not to volatilize.

Due to the distinct UV-absorption together with a significant quantum yield (see B.2.1.9), direct photolysis in air will occur to major extent. Once in the atmosphere rapid photochemical oxidative degradation of bentazone (see B.2.1.10) will occur. Calculation of the atmospheric half-life shows that bentazone will be degraded by reaction with hydroxyl radicals with an estimated half-life of 2.1 hours. A long-range transport via air can therefore be excluded.

Second notifier

Reference is made to the data presented in Bentazone monograph for the first inclusion on Annex I. No changes to the original assessment are relevant

The List of Endpoints gives the following values for physicochemical properties of bentazone relevant for behaviour in air:

Substance	**Bentazone**
Vapour Pressure	1.7e-4 Pa (20°C)

Henry's law constant	7.2e-5 $Pa.m^3.mol^{-1}$
Photochemical half life	It is assumed that the values listed in the LoEP are in fact for water
Photochemical oxidative degradation	DT50: 2.1 h

The rate of photochemical oxidative degradation of bentazone was recalculated according to the model of Atkinson, as implemented in EPISuite v4.

RMS Comment

The second notifier submitted an overview table from the LoEP from the original DAR. Though no changes are considered relevant with respect to the fate and behaviour in air, different values for vapour pressure and Henry's Law constant are reported now by the main notifier. However, the overall concusion with respect to the compartment air does not change.

4.3 Bioaccumulation

4.3.1 Bioaccumulation test on fish

STUDY IIA 8.2.6.1/01 (BASF)

Reference/notifier	:	Anonymous 1992a	GLP statement	:	yes
Type of study	:	fish, bioconcentration	Guideline	:	US EPA 165-4 (1982), OECD 305 E (1981).
Year of execution	:	1991	Acceptability	:	acceptable
Test substance	:	[phenyl-U-^{14} C]-Bentazone (BAS 351 H) radio purity: 97% by HPLC/LSC and unlabelled bentazone (purity > 99%, by HPLC). Water solubility : 0.53 g/L at 25 ºC.	Previous evaluation	:	Submitted for renewal (relevant)

Substance	Species	Duration [d]	Method	BCF [L/kg wwt]	Based on
^{14}C- bentazone	*Lepomis macrochirus*	28 uptake/ 17 depuration	flow-through	1.4	whole fish
				0.4	fillet
				2.2	viscera

Description

Methods. Bluegill sunfish were exposed to ^{14}C-labelled bentazone for 28 days to measure uptake of the compound and then placed in clean water for 17 days to determine elimination rate. Fish were commercially obtained, and acclimated for at least 14 days, mean weight 0.95 g and mean length 34.2 mm at test initiation. Flow-through system, filtered well water hardness 65 mg $CaCO_3$/L and conductivity 203 µmhos/cm. The test solutions were renewed to produce a mean flow rate of 182 L/day (4 tank volumes/day). Prior to initiating the uptake phase of the study, the test solutions were allowed to flow through the test chambers for a 2-day equilibrium phase. One replicate test chamber in the treatment and control group (well water) with 94 fish per chamber. One exposure level at 5 mg/L.

Environmental conditions. Temperature 22 ºC, pH 6.8 -7.2.

Chemical analysis. Stock solution of the test substance was measured on day 0, 7, 14 and 21. Fish samples were taken for tissue analysis at 1, 3, 7, 10, 14, 21 and 28 days into the uptake phase and 1, 3, 7, 10 and 14 days into the depuration phase. On days 0, 21 and 28 fish were sampled from the control group. On each of the fish sampling occasions, four fish were randomly selected from the test vessel. Additional fish were collected on days 10 (8 fish), 28 (20 fish) and 35 (16 fish) from the test solution group for determination of metabolites. Water samples were taken each day during the uptake phase and on day 1 of the depuration phase. Additional water samples on day 0, 10, 28, and 35 for metabolite analysis. Length and weight of fish were recorded after collected. Two fish were analysed on total body radio activity, the other two were used for edible/non-edible analyses. Radioactive residues in the whole body, non edibles (internal organs, carcass, fins) and edible (body, muscles, skin and skeleton) tissues were analysed by combustion in a sample oxidiser and radioactivity was measured by LSC. Tissues were then extracted with a mixture of organic solvents for subsequent determination of residues. Residues were methylated and analysed by radio-TLC. The stock solutions were analysed by radio-TLC, the purity of ^{14}C-bentazone used to prepare the stock solution was analysed by HPLC. Water samples were analysed by radio-TLC after acidification with HCl. LOQ of LSC: 0.13 and 1.3 mg/kg for a sample of 1 g and 0.1 g, respectively. Combustion efficiency of the oxidiser: 98 – 100%.

Calculations and statistics. BCF values for the edible, non-edible and whole body were calculated using kinetic equations estimated from a nonlinear regression (SAS version 6.03). BCF values was also calculated by dividing the mean tissue concentration by the water at steady state on samples days 7 to 28.

Results

No mortality in control during the exposure phase and one fish died in the elimination phase. No abnormal behaviour. The mean concentration of bentazone based on measured radioactivity concentration in the treatment chamber during the exposure phase,was 4.92 mg bentazone/L. Bentazone residues in the whole body were 1.4 times higher than the average concentration in water. Steady state condition was reached after day 7.

The reported BCF values were 0.4, 2.2, and 1.4 L/kg for edible, non-edible and whole body, respectively. No difference between the BCF calculated from kinetic equation and dividing the mean concentration in fish and

water. The half-life time for elimination in edible tissue (23.1 d) was slower the in the non-edible tissue (13.9 d). The half-life time for elimination for whole body was 13.9 d. Only bentazone could be identified.

Remarks by RMS

Molecular formula in applicant's summary report suggests that bentazone-sodium is tested, but reported CAS number and molecular weight is for bentazone. Fish lipid content is not measured and as a result BCF values are not corrected for lipid content. Fish length and weight at the end of the study are not reported. BCF values are also not corrected for growth dilution. However, the results indicate that bioaccumulation of bentazone is low.

4.3.2 Bioaccumulation test with other organisms

4.4 Acute toxicity

4.4.1 Short-term toxicity to fish

B.9.2.1.1 Acute toxicity of the active substance (IIA 8.2.1; IIA 8.3.1; IIA 8.5.1)

Several new studies are submitted, usually they are performed because of data requirements outside the EU, or because the guidelines have been updated. Ownership of the new studies has been indicated.

STUDY IIA, 8.2.1.1/01 (original DAR)

Characteristics

reference	:	Anonymous 1987a.			
type of study	:	Acute toxicity study	species	:	Rainbow trout (*Oncorhynchus mykiss*)
year of execution	:	1987	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	50 and 100 mg/L
guideline	:	OECD 203	dosing method	:	static
test substance		Bentazone (BAS 351 H; Reg. no.: 51 929), batch no. N 187,	acceptability	:	Acceptable
purity	:	97.8%	96-h LC50	:	>100 mg/L

Description

Test item: Bentazone (BAS 351 H; Reg. no.: 51 929), batch no. N 187, purity: 97.8%.

Test species: Rainbow trout (*Salmo Gairdneri* Rich., now called *Oncorhynchus mykiss*); mean body length: 74 mm (65 mm - 82 mm); mean wet weight: 2.6 g (1.6 g - 4.0 g); supplied by Forellenzucht Peter Hofer, 7238 Oberndorf-Aistaig, Germany.

Test design: Static system (96 h); 2 test item concentrations and a water control, 3 replicates for the highest test item concentration and one replicate for the second test item concentration and the control; 10 fish per aquarium (loading 0.26 g fish/L); assessment of mortality and sublethal after 1, 4, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 50 and 100 mg bentazone/L.

Test conditions: Glass aquaria with a stainless steel frame (80x35x46 cm), test volume: 100 L; dilution water: reconstituted freshwater according to DIN 38 412, Part 11, Draft Sept. 1981 prepared from fully demineralized tap water; hardness: 2.5 mmol/L; acid capacity: 0.8 mmol/L; temperature: 12°C; pH : 7.0 - 8.0; oxygen content: 9.8 mg/L - 11.0 mg/L; photoperiod 16 h light : 8 h dark; slight aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method.

Statistics: Descriptive statistics; Probit analysis for calculation of LC_{50}.

Results

Analytical measurements: Analytical verification of bentazone concentrations was conducted in each concentration after 1.5 hours after test initiation and at test termination. The analyzed contents of bentazone ranged from 100.4% to 101.2% of nominal after 1.5 hours after test initiation and from 100.2% to 101.4% at test termination. The following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the water control and the test item concentration of 50 mg a.s./L, whereas 10% mortality was observed at the highest tested concentration of 100 mg a.s./L. No sub-lethal effects were found in the control groups and in all test item treatments after 96 hours. The results are summarized in B.9.2.1.1-01.

Table B.9.2.1-01: Acute toxicity (96 h) of bentazone to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L] (nominal)	**Control**	**50**	**100**
Mortality [%] (96 h)	0	0	10
Symptoms (after 96 h)	none	none	none
Endpoints [mg bentazone/L] (nominal)			
LC_{50} (96 h)	> 100		
NOEC (96 h)	50		

Conclusion

96-hour LC50 >100 mg/L.

STUDY IIA, 8.2.1.2/01

Characteristics

reference	:	Anonymous 1986a			
type of study	:	Acute toxicity study	species	:	Bluegill (*Lepomis macrochirus)*
year of execution	:	1986	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	50 and 100 mg/L
guideline	:	OECD 203	dosing method	:	static
test substance		Bentazone (BAS 351 H; Reg. no.: 51 929), batch no. MS 2 F 22,	acceptability	:	Acceptable
purity	:	94%	96-h LC50	:	>100 mg/L

Description

Test item: Bentazone (BAS 351 H, Reg. no. 51 929, batch no. MS 2 F 22): purity 94.0%.

Test species: Bluegill (*Lepomis macrochirus* RAF.), body length: 4.2 cm (3.9 - 4.8 cm); body weight: 0.9 g (0.7 - 1.3 g); supplied by "Hazleton Research Products, Inc.", Denver, PA, USA).

Test design: Static system (96 hours); 10 fish per aquarium, 2 test item concentrations and a water control, 3 replicates for the highest test item concentration and one replicate for the second test item concentration and the control; loading rate: about 0.2 g/L; assessment of mortality and symptoms of toxicity after 1, 4, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 50, 100 mg bentazone/L).

Test conditions: Glass aquaria with a stainless steel frame (60x35x40 cm), test volume: 50 L; dilution water: reconstituted freshwater according to DIN 38 412, Part 11, Draft Sept. 1981 prepared from fully demineralized tap water; hardness: 2.5 mmol/L; acid capacity: 0.8 mmol/L; temperature: 23°C; pH : 7.4 - 8.2; oxygen content: 8.1 mg/L - 8.4 mg/L; photoperiod 16 h light : 8 h dark; continuous slight aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method.

Statistics: Descriptive statistics, probit analysis for calculation of LC_{50}.

Results

Analytical measurements: Analytical verification of bentazone concentrations was conducted in each concentration 1 hour after test initiation and at test termination. The analyzed contents of bentazone ranged from 97.2% to 100.3% of nominal 1 hour after test initiation and from 100.2% to 101.1% at test termination. As measured concentrations confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the water control and test item concentrations up to and including the highest test item concentration of 100 mg a.s./L. No sub-lethal effects were found in the control group and at all test item treatments after 96 hours. The results are summarized in B.9.2.1.1-02.

Table B.9.2.1.1-02: Acute toxicity (96 h) of bentazone to bluegill (*Lepomis macrochirus*)

Concentration [mg a.s./L] (nominal)	**Control**	**50**	**100**
Mortality [%] (96 h)	0	0	0
Symptoms (after 96 h)	none	none	none
Endpoints [mg bentazone/L] (nominal)			
LC_{50} (96 h)	> 100		
NOEC (96 h)	≥ 100		

Conclusion

96-hour LC50 >100 mg/L.

STUDY IIA, 8.2.1.2/02

Characteristics

reference	:	Anonymous 1983			
type of study	:	Acute toxicity study	species	:	Common carp (*Cyprinus carpio*)
year of execution	:	1986	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	180, 320, 560 and 1000 mg/L
guideline	:	OECD 203	dosing method	:	static
test substance		Bentazon-Na; BAS 351 H; test substance no.: 83/115.	acceptability	:	Acceptable
purity	:	100%	96-h LC50	:	>1000 mg/L

Description

Test item: Bentazon-Na; BAS 351 H; test substance no.: 83/115.

Test species: Common carp (*Cyprinus carpio*), body length: 5.5 cm (4.6 - 6.3 cm); body weight: 2.5 g (1.3 - 3.5 g); supplied by Saitama pref. breeding fishery co-op 1060, Kitakohama, OH-AZA Kazo City, Saitama Pref.

Test design: Static system (96 h); 4 test item concentrations plus a dilution water control, 1 replicate per treatment; 10 fish per aquarium (loading 0.5 g fish/L); assessment of mortality and sublethal effects after 1, 4, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 180, 320, 560 and 1000 mg bentazon-Na/L (nominal).

Test conditions: Glass aquaria with a stainless steel frame (60x35x40 cm), test volume: 50 L; dilution water: reconstituted freshwater according to DIN 38 412, Part 11, Draft Sept. 1981 prepared from fully demineralized tap water; hardness: 2.5 mmol/L; acid capacity: 0.8 mmol/L; temperature: 25°C; pH: 7.7 - 8.2; oxygen content: 7.7 mg/L - 8.3 mg/L; photoperiod 16 h light : 8 h dark; continuous slight aeration.

Analytics: Analytical verification of test item concentrations was conducted using a UV-spectral photometry method.

Statistics: Descriptive statistics; probit analysis for calculation of LC_{50}.

Results

Analytical measurements: Analytical verification of bentazon-Na concentrations was conducted in each concentration after 1 h, 24 h, 48 h, 72 h and at the end of the test (96 h). The analyzed contents of bentazon-Na ranged from 101% to 102%, 102% to 103%, 102% to 104% and 101% to 102% after 1 h, 24 h, 48 h and 72 h, respectively. At test termination, measured contents of bentazon-Na were between 100% and 101% of nominal. As measured concentrations confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control and at test item concentrations of up to and including the highest test item concentration of 1000 mg a.s./L. No sub-lethal effects were found in the control group and at all test item treatments after 96 hours. The results are summarized in B.9.2.1.1-03.

Table B.9.2.1.1-03: Acute toxicity (96 h) of bentazon-Na to common carp (*Cyprinus carpio*)

Concentration [mg a.s./L] (nominal)	**Control**	**180**	**320**	**560**	**1000**
Mortality [%] (96 h)	0	0	0	0	0
Symptoms (after 96 h)	none	none	none	none	none
Endpoints [mg bentazon-Na/L] (nominal)					
LC_{50} (96 h)	> 1000				
NOEC (96 h)	≥ 1000				

Conclusion

96-hour LC50 >1000 mg/L.

STUDY IIA 8.2.1.2/03 (BASF)

Reference/notifier :	Anonymous. (2011a)	GLP statement :	yes
Type of study :	fish, acute toxicity	Guideline :	OPPTS 850.1075; EPA 540/9-82-024, 72-1, OECD 203 (1992)
Year of execution :	2011	Acceptability :	Acceptable
Test substance :	Technical Reg no 88691: bentazone-sodium (BAS 351 H-Na), batchCOD-001417, purity 91.9 % (analysed), appearance yellowish solid	Previous evaluation :	Submitted for renewal (relevant)

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg/L]
bentazone-sodium	*Pimephales promelas*	static	23	8.2-8.4	96	LC_{50}	> 113.5

Description

Methods. Toxicity of bentazone-sodium to fathead minnow (*Pimephales promelas*) was determined under static conditions. Fish were bred and hatched at the testing facility and acclimated in the testing conditions for at least 14 days. Fish were approximately 2 months old, length of control fish 1.5 – 2.2 cm and mean wet weight 0.06 – 0.17 g. Loading 0.12 g/L.Limit test at 120 mg/L nominal (110 mg bentazone-sodium/L after correction for purity) and a control. Dilution with non-chlorinated charcoal filtered tap water mixed with deionised water, total hardness 100 mg $CaCO_3$/L, conductivity 250 µS/cm, pH 7.5 – 8.5. Two replicates for the control and three in the treatment concentration with 10 fish each, 10 L water per test vessel. Observations within 1 hour after the start, 6, 24, 48, 72 and 96 h after test initiation.

Conditions. 16:8 h L:D (96 - 451 lux), no aeration, no feeding.

Chemical analysis. Samples were collected at beginning, after 48 hours and at the end of the test. Analysis by HPLC with UV detection at 230 nm after acidification with sulphuric acid and dilution with acetonitrile/water. The method was validated with spiked samples which were analysed directly after preparation, and after a storage period of 4 days. Recovery in HPLC analysis for directly measured samples was 93.9 – 102.9% of nominal concentrations. No degradation was observed in stored samples, recovery ranged from 95.7 – 105.3% of nominal. LOQ: 1 mg/L.

Calculations and statistics. NOEC was determined by visual interpretation of mortality data.

Results

Measured concentrations were 110-113 mg/L (99.8 – 102.3%) at the start , 112-113 mg/L (101.9 – 102.3%) after 48 hours and 116-117 mg/L (105.1 – 105.7%) at test termination. Mean measured concentration was 113.5 mg/L (expressed as pure bentazone-sodium; 103% of nominal). No mortality in controls and at 120 mg/L after 96 hours. No adverse effects or abnormal behaviour were observed. LC_{50} is reported as > 113.5 mg/L, based on mean measured concentrations.

Remarks by RMS

Water quality parameters within accepted limits. The result 96-hours LC_{50} > 113.5 mg/L, based on mean measured concentrations of bentazone-sodium, is used for risk assessment.

STUDY IIA 8.11.1/01 (BASF)

Reference/notifier :	Graves, W.C. and Smith G.J. (1991a)	GLP statement	: yes
Type of study :	fish, acute toxicity	Guideline	: ASTM E 729-88; EPA FIFRA-E 540/9-82-024 (1982)
Year of execution :	1991	Acceptability	: acceptable
Test substance :	Bentazone (BAS 351 H-tech. a.i.), batch 68-901102, purity 53.0 %, appearance dark amber liquid, water solubility: 500 mg/L at 20 ºC.	Previous evaluation	: Submitted for renewal (relevant)

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg/L]
bentazone	*Cyprinodon variegatus*	flow-through	21.8-22.2	8.2-8.4	96	LC_{50}	> 136

Description

Methods. The acute toxicity of bentazone to sheepshead minnow (*Cyprinodon variegatus)* was tested under flow-through conditions. Juvenile fish were reared in own laboratory facilities. Fish were held under test conditions for 14 days and acclimated for 50 h prior to testing. Length of control fish 23 – 30 mm and mean wet weight 0.31– 0.96 g at end of the test. Loading 0.90 g/L. A stock solution was prepared in deionised water and mixed with salt water. Nominal concentration 120 mg/L (corrected for purity of the test compound) and a salt water control. Dilution with natural sea water which was passed through a sand filter and aerated, salinity 25 $^{o}/_{oo,}$ pH 8.3. Three replicates with 10 fish each, 6.5 L water per test vessel, renewal rate 7.8 volumes/24 hours. Observations 4, 24, 48, 72 and 96 h after test initiation.

Conditions.: 22 ± 1 ºC, 16:8 h L:D (986 lux, with 30 minutes transition period), no aeration, no feeding 48 h prior to testing and during the test.

Chemical analysis. Samples were collected at beginning and at 24 h intervals. Analysis by GC with a specific detector after acidification to pH 2 and extraction with methylene chloride, LOD 1.0 mg as/L. Mean procedural recovery was 111% and 104% at fortifications of 1.0 and 120.0 mg/L, respectively. Overall procedural recovery was 109%.

Calculations and statistics. LC_{50}-values were determined using the program of Stefan (1977) using the probit analysis and visual interpretation.

Results

Measured concentrations of bentazone (corrected for mean procedural recovery) were 118.3 – 127.9 mg/L at the start and 137.5 – 146.2 mg/L at the end. Mean measured concentrations ranged from 102 to 127% of nominal, overall mean measured concentration was 136.0 mg/L. No mortality in control, and at the test concentration of 136.0 mg/L. All fish appeared normal. 96-hours LC_{50} > 136.0 mg/L based on mean measured test concentration.

Remarks by RMS

Water quality parameters within accepted limits. The analytical measured test concentration was above the range of 120% of nominal. The result 96-hours LC_{50} > 136.0 mg/L, based on mean measured test concentration, is used for risk assessment.

4.4.2 Short-term toxicity to aquatic invertebrates

STUDY IIA, 8.3.1.1/05

Characteristics

reference	:	Bias 1986a			
type of study	:	Acute toxicity study	species	:	*Daphnia magna*
year of execution	:	1986	exposure duration	:	48 hours
GLP statement	:	no	nominal concn.	:	62.5, 125, 250, and 500 mg/L
guideline	:	OECD 202	dosing method	:	Static
test substance		Bentazone, Reg. No. 519 29, batch no. MS 2 F 22	acceptability	:	Not acceptable
purity	:	94%	48 h-EC50	:	125 mg/L

Description

Test item: Bentazone, Reg. No. 519 29, purity: 94.0%; batch no. MS 2 F 22.

Test species: Water flea (*Daphnia magna* STRAUS), neonates from in-house culture, 6 -24 h of age; (originally obtained from Institute National de Recherche Chimique Appliquée, France).

Test design: Static system (48 hours), 4 test concentrations plus dilution water control, 4 replicates with 5 daphnids in each; assessment of immobility at test initiation and after 3, 6, 24 and 48 hours.

Endpoints: EC_{50} and NOEC based on immobility of daphnids.

Test concentrations: Control (dilution water), 62.5, 125, 250 and 500 mg bentazone/L (nominal).

Test conditions: 250 mL glass beakers, test volume 200 mL, dilution water: tap water, purified by charcoal and filtered through a 6 µm filter plus deionized water; temperature: 292.0 - 294.0 K; pH 3.32 - 8.19; oxygen content: 8.60 mg/L - 9.52 mg/L; conductivity: 500 - 650 µS/cm; total hardness: 2.70 ± 0.50 mmol/L; acid capacity: 0.80 ± 0.10 mmol/L; photoperiod: 16 hours light : 8 hours dark; light intensity: < 5 µEinstein/m^2 s^1; no feeding; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method.

Statistics: Descriptive statistics; Spearman-Kaerber estimation for determination of EC_{50}.

Results

Biological results: After 48 h of exposure, no immobility of daphnids was observed in the control and at the test item concentration of 62.5 mg a.s./L, whereas 50% of the daphnids were immobile at 125 mg a.s./L. At the two highest test item concentrations of 250 and 500 mg a.s./L all daphnids were found immobile after 48 h of exposure. However in the two highest test item concentrations the pH-values were extremely low (less than 4). Therefore it cannot be ruled out, that the registered mortality must at least partially be attributed to the corresponding acidity. For results see Table B.9.2.1.1-08.

Table B.9.2.1.1-04: Effect of bentazone on *Daphnia magna* immobility

Concentration [mg a.s./L] (nominal)	**Control**	**62.5**	**125**	**250**	**500**
Immobility (24 h) [%]	0	0	5	100	100
Immobility (48 h) [%]	0	0	50	100	100
	Endpoints [mg bentazone/L] (nominal)				
EC_{50} (48 h)	125 (95% confidence limits: 106.96 - 146.08)				
NOEC = EC_0 (48 h) [+]	62.5				

[+] EC_0 is the highest concentration tested with an effect ≤ 10%.

Conclusion

48-hour EC50 125 mg/L.

STUDY IIA 8.3.1.1/01 (BASF)

Reference/notifier	: Jatzek, H.J. (2003b)	GLP statement	: yes
Type of study	: Daphnia, acute toxicity	Guideline	: OECD 202 (1984), EPA-OPPTS 850.1010
Year of execution	: 2003	Acceptability	: acceptable
Test substance	: BAS 351 H (bentazone), purity: 98.4% (analysed); yellowish solid, batch no: 14-7887	Previous evaluation	: Submitted for renewal (relevant)

Substance	Species	Method	T	pH	Duration	Criterion	Value
			[°C]		[h]		[mg/L]
bentazone	*Daphnia magna*	static	18 - 22	7.5 – 8.1	48	LC_{50}	> 100

Description

Methods. Daphnids (< 24 h old) were exposed to bentazone for 48 h in a static test (loading 0.1 mL/animal). Reconstituted M4 synthetic fresh water, total hardness 2.44 mmol/L, ratio Ca:Mg 4:1; pH 8.1, conductivity 608 µS/cm. Nominal concentrations 12.5, 25, 50, 100 mg/L, and a control. Four replicates for control and test compound, 5 daphnids per test unit. Three additional replicates per test concentration and control, one for measurement of pH and oxygen at the start of the study and two for analysis of test concentration. Observations of immobility and clinical signs of toxicity were made at 0, 24 and 48 h after test initiation. A positive control was performed with potassium dichromate two days before.

Conditions. no aeration, 16:8 h L:D (1 – 8 µE/m^2.s at 400 – 750 nm), no feeding.

Analytical methods. Samples were collected at 0, and 48 h after test initiation and analysed by HPLC with UV detection at 210 nm.

Calculations and statistics. Statistical calculation was not necessary.

Results

Recovery was 103.2 – 104.0% of nominal at test initiation and 101.5 – 102.8% of nominal at end. One daphnid was found immobile in the control and one in the lowest test concentration of 12.5 mg/L. No sublethal effects were observed. Nominal 48-hours EC_{50} reported as > 100 mg/L. EC50 (24 h) for potassium dichromate is reported to be 1.09 mg/L.

Remarks by RMS

Water quality parameters within accepted range. Validation of the analytical method is not reported. The 48-h EC_{50} for immobility of > 100 mg/L based on nominal concentrations is used for risk assessment.

STUDY IIA 8.11.1/02 (BASF)

Reference/notifier	:	Graves, W.C. and Smith G.J. (1991a)	GLP statement	:	yes
Type of study	:	mysid shrimp, acute toxicity	Guideline	:	EPA-E 540/9-82-024, ASTM E 729-88
Year of execution	:	1991	Acceptability	:	acceptable
Test substance	:	Bentazone (BAS 351 H-Tech. A.I.), batch 68-901102, purity 53.0%, appearance dark amber liquid. Water solubility 500 mg/L at 20 ºC.	Previous evaluation	:	Submitted for renewal (relevant)

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg/L]
bentazone	*Mysidopsis bahia*	flow-through	24.76-25.0	8.2-8.3	96	LC_{50}	>132.5

Description

Methods. The acute toxicity of bentazone to juvenile *Mysidopsis bahia* was tested under flow-through conditions. Mysids were reared at Wildlife International Ltd. and held under test conditions for at least 14 days. A stock solution was prepared in deionised water and mixed with salt water. Nominal concentration 120 mg/L (corrected for purity of

the test compound), and a salt water control. Dilution with natural sea water which was passed through a sand filter and aerated, salinity 25 $^{o}/_{oo,}$ pH 8.1. Three replicates with 10 organisms each, 6.5 L solution per unit, flow-rate 7.8 volume additions of test water per day. Observations on mortality, signs of toxicity and abnormal behaviour were performed 4, 24, 48, 72 and 96 h after test initiation.

Conditions. 25 ± 1 ºC, 16:8 h L:D (986 lux), no aeration, daily feeding with live brine shrimp.

Chemical analysis. Daily samples to determine actual concentrations. Analysis by GC with a specific detector after acidification to pH 2 and extraction with methylene chloride, LOD 1.0 mg/L. Mean procedural recovery of was 107% and 98.3% at fortifications of 1.0 and 120.0 mg/L, respectively. Overall procedural recovery was 112%.

Calculations and statistics. 96-h LC_{50}-values and 95% confidence limits were calculated using probit method of calculation according to Stefan (1977).

Results

Measured concentrations of bentazone (corrected for mean procedural recovery) were 97.6 – 202.4 mg/L at the start and 109.8 – 156.6 mg/L at the end. Mean measured test concentrations over the 96 h ranged from 101 to 120% of nominal, overall mean measured test concentration was 132.5 mg/L. One mortality in the both control and the treatment after 72 hours of exposure. All surviving mysids appeared normal. 96-hours LC_{50} reported as > 132.5 mg/L, based on mean measured concentrations.

Remarks by RMS

Water quality parameters within accepted range. The analytical measured test concentration at some sample times were far above 120% of nominal, especially after 72 and 96 hours of exposure when the highest concentrations were 213.6 and 156.6 mg/L (178% and 130% of nominal), respectively. The result 96-h LC_{50} > 132.5 mg as/L, based on mean measured concentrations, is used for risk assessment.

STUDY IIA 8.11.1/03 (BASF)

Reference/notifier	:	Graves, W.C. and Smith, G.J. (1992a)	GLP statement	:	yes
Type of study	:	oyster embryo, acute toxicity	Guideline	:	ASTM E 729-88, EPA 540/9-82-024
Year of execution	:	1992	Acceptability	:	acceptable
Test substance	:	Bentazone (BAS 351H) batch 68-901102, purity 53 %, appearance dark amber liquid. Water solubility 500 mg/L at 20 ºC.	Previous evaluation	:	Submitted for renewal (sublementary)

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg/L]
bentazone	*Crassostrea virginica*	flow-through	21.6-22.0	7.9-8.1	96	EC_{50}	>109

Description

Methods. The effects of bentazone on shell deposition of the eastern oyster, *Crassostrea virginica,* were tested under flow-through conditions. Oysters were obtained from a commercial company and were held under test conditions for at

least 10 days prior to test initiation. Length of the oysters was 35-40 mm (mean 47 mm). Nominal concentrations 15.6, 25.9, 43.2, 72.0, and 120 mg/L (corrected for purity of the test compound) and a control (salt water). Dilution with filtered natural seawater, salinity 22 ‰, pH 7.8. Prior to introduction of the oysters, 1 – 7 mm of the shell was ground off. Observations for clinical signs of toxicity and mortality were made twice daily. At test termination mean shell deposition was calculated by measuring the longest finger of growth for each oyster with calipers. Shell deposition was expressed as percent inhibition of mean shell growth for each treatment relative to control shell growth. One replicate with 20 oysters each, 12.6 L water per test vessel, renewal rate 1 L/oyster/h.

Conditions. 22 ± 1 ºC, 16:8 h L:D (30 min. transition period) 409 lux, no aeration, feeding with an algal suspension during holding and throughout the test.

Chemical analysis. Samples were collected at beginning and at 24 h intervals. Analysis by GC with a specific detector after acidification to pH 2 and extraction with methylene chloride, LOD 1.0 mg/L. Mean procedural recovery was 83-86% and 96.7 - 124% at fortifications of 10 and 120.0 mg/L, respectively. Overall procedural recovery was 98%.

Calculations and statistics. EC_{50}-value was calculated according to Stephan (1977) using probit method. The observed effect concentration was determined by visual inspection of the data.

Results

Mean measured concentrations (corrected for mean procedural recovery) were 10, 19, 29, 61 and 109 mg bentazone/L (64 - 91 % of nominal). No mortalities occurred in any treatment. Shell deposition was 4.10 mm in the control. Percent inhibition was 30.7, 22.4, 31.2, 28.8, and 38.3% at 10, 19, 29, 61, and 109 mg/L. No clear dose-response relationship was found, but individual oyster shell growth measurements indicated that the differences from control in all treatment groups were most likely treatment related. The 96-EC_{50} is reported as > 109 mg/L, the NOEC based on visual inspection is reported to be < 10 mg/L, both values based on mean measured concentrations.

Remarks by RMS

Water quality parameters within accepted range. The result EC_{50} > 109 mg/L, based on mean measured concentrations, is used for risk assessment.

4.4.3 Algal growth inhibition tests

STUDY IIA, 8.4/03

Characteristics

reference	:	Dohmen (1990a)		:	
type of study	:	Algae growth inhibition	species	:	*Ankistrodesmus bibraianus* (now known as *Selenastrum bibraianum* Reinsch)
year of execution	:	1990	exposure duration	:	72 hours
GLP statement	:	Yes	nominal concn.	:	0.5, 2.5, 5.0, 10.0, 25.0, 50.0, 75.0 and 100mg/L
guideline	:	OECD 201	dosing method	:	Static; stock solution in test water
test substance	:	Reg. no. 5178870	acceptability	:	Not acceptable

		(M650F03), HCl salt, batch L71-148			
purity	:	82.6% (96.2% for HCl salt)	72h-EbC50	:	62 mg/L

Description

Test item: Bentazone (BAS 351 H; Reg. No. 51 929), batch no. 90-1, purity: 98.4%.

Test species: Fresh water green alga, *Ankistrodesmus bibraianus* (now known as *Selenastrum bibraianum* Reinsch), stock obtained from the "DSM Collection" Göttingen, Germany.

Test design: Static system; test duration 72 hours; 8 test concentrations plus a control, each with 5 replicates per treatment; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to biomass after exposure over 72 hours.

Test concentrations: Control, 0.5, 2.5, 5.0, 10.0, 25.0, 50.0, 75.0 and 100.0 mg BAS 351 H/L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 50 mL; nutrient solution according to OECD 201; pH 8.04 at test initiation and pH 7.75 - 7.91 at test termination; temperature: 21 °C ± 1 °C; initial cell densities: 4 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking at 125 rpm.

Analytics: No analytical verification of test item concentrations was conducted.

Statistics: Descriptive statistics; graphic determination of EC_x values for biomass.

Results

Analytical measurements: No analytical verification of test item concentration was conducted. The following biological results are based on nominal concentrations.

Biological results: The effects on algal growth (biomass) are summarized in B.9.2.1.1-09.

Table B.9.2.1.1-05 Effect of bentazone on the growth of green alga *A. bibraianus*

Concentration [mg/L] (nominal)	**Control**	**0.5**	**2.5**	**5.0**	**10.0**	**25.0**	**50.0**	**75.0**	**100.0**
Inhibition in 72 h (biomass) [%]	--	2.8	14.3	6.9	22.3	43.5	49.2	61.1	61.7
Endpoints [mg bentazone/L] (nominal)									

Concentration [mg/L] (nominal)	**Control**	**0.5**	**2.5**	**5.0**	**10.0**	**25.0**	**50.0**	**75.0**	**100.0**
E_bC_{50} (72 h)	62.0								
E_bC_{10} (72 h)	≈ 1.5								

Conclusion

An EbC50 of 62.0 mg/L is determined. As the tested dose rates were not analytically confirmed and as the test guideline is not up to date anymore, this value cannot be used for risk assessment.

STUDY IIA, 8.4/04

Characteristics

reference	:	Dohmen (1990b)		:	
type of study	:	Algae growth inhibition	species	:	*Ankistrodesmus bibraianus* (now known as *Selenastrum bibraianum* Reinsch)
year of execution	:	1990	exposure duration	:	72 hours
GLP statement	:	Yes	nominal concn.	:	1.0, 2.5, 5.0, 10.0, 25.0, 35.0, 50.0, 75.0 and 100mg//L
guideline	:	OECD 201	dosing method	:	Static
test substance	:	Reg. no. 5178870 (M650F03), HCl salt, batch L71-148	acceptability	:	Not Acceptable
purity	:	82.6% (96.2% for HCl salt)	72h-EbC50	:	71 mg/L

Description

Test item: Bentazone-DEA-salt (CAS-No.: 54792-07-3), batch no. WH6256, PCP 00560, purity: 76% (corresponding to 53% bentazone (BAS 351 H, Reg. No. 51 929)).

Test species: Fresh water green alga, *Ankistrodesmus bibraianus* (now known as *Selenastrum bibraianum* Reinsch), stock obtained from the "DSM Collection" Göttingen, Germany.

Test design: Static system; test duration 72 hours; 9 test concentrations plus a control, each with 5 replicates per treatment; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to biomass after exposure over 72 hours.

Test concentrations: Control, 1.0, 2.5, 5.0, 10.0, 25.0, 35.0, 50.0, 75.0 and 100.0 mg bentazone/L (nominal); corresponding to 1.44, 3.6, 7.2, 14.4, 36.0, 50.4, 72.0, 108.0 and 144.0 mg bentazone-DEA-salt/L. The calculations are made on the basis of a content of bentazone of 53% in the test substance.

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 50 mL; nutrient solution according to OECD 201; pH 7.9 at test initiation and pH 7.54 - 7.72 at test termination; temperature: 21 °C ± 1 °C; initial cell densities: 4 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking at 125 rpm.

Analytics: No analytical verification of test item concentrations was conducted.

Statistics: Descriptive statistics; graphic determination of EC_x values for biomass.

Results

Analytical measurements: No analytical verification of test item concentration was conducted. The following biological results are based on nominal concentrations.

Biological results: The effects on algal growth (biomass) are summarized in B.9.2.1.1-10.

Table B.9.2.1.1-6Effect of bentazone [*] on the growth of green alga *A. bibraianus*

Concentration of bentazone [mg/L] (nominal)	**Control**	**1.0**	**2.5**	**5.0**	**10.0**	**25.0**	**35.0**	**50.0**	**75.0**	**100.0**
Concentration of bentazone-DEA-salt [mg/L] (nominal)	**Control**	**1.44**	**3.6**	**7.2**	**14.4**	**36.0**	**50.4**	**72.0**	**108.0**	**144.0**
Inhibition in 72 h (biomass) [%] [#]	--	5.3	-2.4	-0.5	23.1	25.0	23.6	40.4	61.0	60.9
	Endpoints [mg bentazone/L] (nominal)					**Endpoints [mg bentazone-DEA-salt/L] (nominal)**				
E_bC_{50} (72 h)	71					102				
E_bC_{10} (72 h)	5.0					7.2				

* applied as bentazone-DEA-salt

[#] Negative values indicate stimulated growth compared to the control.

Conclusion

An EbC50 of 62.0 mg/L is determined. As the tested dose rates were not analytically confirmed and as the test guideline is not up to date anymore, this value cannot be used for risk assessment.

STUDY IIA 8.4/01 (BASF)

Reference/notifier	: Jatzek, H.-J. (2003b)	GLP statement	: yes
Type of study	: algae, growth inhibition	Guideline	: OECD 201 (1984), EPA OPPTS 850.5400
Year of execution	: 2003	Acceptability	: acceptable
Test substance	: BAS 351 H (bentazone), purity 98.4%; Solid/yellowish	Previous evaluation	: Submitted for renewal (relevant)

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg//L]
Bentazone	*Pseudokirchneriella subcapitata*	static	23		72	E_rC_{50}	33.3
						E_bC_{50}	16.8

Description

Methods. Static growth inhibition test with bentazone, nominal concentrations of 6.25, 12.5, 25, 50, and 100 mg/L and a control. Three replicates for test substance and five in the control. 100 mL test solution per test unit, initial cell density 1 x 10^4 cells/mL. An additional vessel without algae for analytical measurements. Cells were counted after 0, 24, 48, and 72 h by in-vivo chlorophyll fluorescence at 435 nm and counted under a microscope.

Chemical analysis. Samples were taken at the start and at the end, analysis by HPLC with UV detection at 210 nm.

Conditions. Continuous light (60 – 120 µE/(m^2.s).

Calculations and statistics. Growth rate and biomass were determined according to the guideline. EC values were estimated with linear regression analysis from the concentration-response relationship.

Results

Actual concentrations ranged from 101.2% to 103.8% of nominal at the start and from 102.4% to 104.0% of nominal at the end. Test results were based on nominal concentrations. Cell numbers in the control after 72 h were 208 x 10^4 cells/mL (109 fold), growth was exponential. A dose-related inhibition of growth rate and biomass was found. Growth rate was reduced by 96.1% at 100 mg/L and biomass by 99.3% at 100 mg/L. The E_rC_{50} is reported as 33.3 mg/L, and E_bC_{50} 16.8 mg/L based on nominal concentrations. The E_rC_{10} was 9.89 mg/L and the E_bC_{10} 7.90 mg/L.

Remarks by RMS

Water quality parameters were within acceptable values. Control meets validity criteria of current guideline version. Report is only a brief description of the study, no validation was reported for the analytical method and no description of the statistical evaluation. It is not clearly reported whether the cells are kept in suspension by constant shaking. The result 72-h E_rC_{50} 33.3 mg/L and E_bC_{50} 16.8 mg/L based on nominal concentrations are used for risk assessment.

4.4.4 Lemna sp. Growth inhibition tests

STUDY IIA, 8.6/06

Characteristics

Reference	:	Hughes . (1991a);	Species	:	*Lemna gibba*
Type of study	:	Lemna, growth inhibition	Exposure duration	:	7 days
Year of execution	:	1991	Nominal concn.	:	1, 2, 4, 8 and 16 mg/L
GLP statement	:	Yes	Dosing method	:	Static
Guideline	:		Acceptability	:	Acceptable
Test substance	:	Bentazone (BAS 351 H, Reg. no. 51 929, lot no. 68-901102)	72h-EyC50	:	5.35 mg/L
Purity	:	53%			

Description

Test item: Bentazone (BAS 351 H, Reg. no. 51 929, lot no. 68-901102): purity 53.0%.

Test species: Duckweed (*Lemna gibba* G3), inocula 7 days old cultures; cultures maintained in-house; stock obtained from "Smithsonian Institution Radiation Biology Laboratory", Rockville, USA.

Test design: Static system (14 days); 6 treatment groups (5 test item concentrations, control) with 3 replicates for the test item treatments and the control; 3 plants with 4 fronds, total number of fronds at test initiation: 12 per replicate; assessment of growth (frond number) on days 2, 4, 7, 9, 11 and 14.

Endpoints: EC_{50} with respect to yield after exposure over 14 days.

Test concentrations: Control, 1, 2, 4, 8 and 16 mg bentazone/L (nominal), corresponding to mean measured concentrations of <0.10, 0.794, 1.53, 3.06, 6.48 and 13.75 mg bentazone/L.

Test conditions: 500 mL Erlenmeyer flasks, test volume 200 mL, synthetic 20x-AAP nutrient medium, pH 7.61 - 7.71 at test initiation and pH 8.63 - 9.59 at test termination; temperature: 25 ± 2 °C, continuous light, light intensity: 4198 - 5813 lumens/m^2.

Analytics: Analytical verification of test item concentrations was conducted using a GC-method with nitrogen-phosphorus detection.

Statistics: Descriptive statistics, weighted least squares nonlinear regression for calculation of the EC_{50} values, ANOVA followed by Dunnett's test for determination of the NOEC value ($\alpha = 0.05$).

Results

Analytical measurements: Analytical verification of test item concentrations was conducted in each test item concentration at the beginning and at the end of the test. Mean measured values for bentazone ranged from 88% to 96% of nominal concentrations at test initiation and from 57% to 79% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 12 fronds per vessel to an average of 736 fronds per vessel after 14 days, corresponding to a 61.3 x multiplication. After 14 days of exposure, statistically significant differences in yield compared to the control were observed at the two highest test item concentrations of 6.48 and 13.75 mg a.s./L (ANOVA followed by Dunnett's test, $\alpha = 0.05$). Effects on yield are summarized in Table B.9.2.1.1-07.

Table B.9.2.1.1-07: Effect of bentazone on the growth of duckweed *Lemna gibba*

Concentration [mg a.s./L] (nominal)	**Control**	**1**	**2**	**4**	**8**	**16**
Concentration [mg a.s./L] (mean measured)	**--**	**0.794**	**1.53**	**3.06**	**6.48**	**13.75**
Inhibition after 14 d [%] [#] (yield based on frond no.)	--	2.9	-2.8	11.6	66.5 *	94.7 *
Endpoints [mg bentazone/L] (mean measured)						
E_yC_{50} (14 d) based on frond no.	5.35 (95% confidence limits: 4.85 - 5.90)					
NOEC (14 d)	3.06					

[#] Negative values indicate stimulated growth compared to the control.

* Statistically significantly different compared to the control (ANOVA followed by Dunnett's test, $\alpha = 0.05$).

Conclusion

14-d EyC50 5.35 mg/L.

STUDY IIA 8.6/01 (BASF)

Reference/notifier	:	Hoffmann, F. (2011b)	GLP statement	:	Yes
Type of study	:	Lemna, growth inhibition	Guideline	:	OECD 221
Year of execution	:	2011	Acceptability	:	Acceptable
Test substance	:	Bentazone (Reg no 51929) batch nr.COD-001416, purity 100%, solid.	Previous evaluation	:	Submitted for renewal (relevant)

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [mg/L]

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [mg/L]
Bentazone	*Lemna gibba*	static	24 - 25	7.48 – 8.45	7	ErC_{50}	12.0
						EyC_{50}	7.1

Description

Methods. Toxicity of bentazone to *Lemna gibba* was assessed under static conditions by exposure through the water phase. Nominal test concentrations 0.41, 1.23, 3.70, 11.1, 33.3, and 100 mg/L, and culture medium control. Test medium was 20X AAP medium according to OECD guideline, pH adjusted to 7.5. Test units contained 160 mL test medium, three replicates for the test treatments and six for the control. Two plants with four fronds and one plant with three fronds per replicate (total 11 fronds/replicate). Number of fronds was determined on days 3, 5 and 7 and observations on chlorosis, necrosis, chlorosis, changes in plant size of shape and root growth were made. In addition the biomass based on dry weight was determined at test initiation and at test termination.

Conditions. Temperature controlled incubator at 24 – 25 ºC, continuous light (8300 lux).

Chemical analysis. Samples were taken at the start and at the end, analysis by HPLC-UV (230 nm) after dilution with acetonitrile/water mixture and acidification with sulfuric acid, method recovery 99.8 – 102.4% and 95.7 – 103.0% for fresh and aged fortified samples, respectively. LOQ: 0.1 mg/L.

Calculations and statistics. Mean plant and frond counts were used to calculate the percent inhibition. Numbers of plants and fronds, and dry weight was evaluated by probit analysis using the Chi-square test. Statistical program used was TOXRAT Professional version 2.10.

Results

Measured concentrations ranged from 99.0% to 105.1% of nominal at the start and from 78.4% to 104.8% of nominal at the end. Geometric mean measured concentrations were 0.36, 1.14, 3.5, 11.1, 34.2, and 103.7 mg/L, respectively. frond numbers in the control showed a 12.2-fold multiplication in seven days. Mean number of fronds after seven days was 134.0 in control and 138.0, 137.3, 128.7, 54.3, 28.0, and 21.7 at 0.41, 1.23, 3.7, 11.1, 33.3, and 100 mg/L, respectively. A summary of inhibition percentages is presented below.

Table B.9.2.1.1-10. Inhibition of growth rate and yield of *Lemna gibba* after 7 days exposure to bentazone.

Nominal concentration [mg/L]	Mean measured concentration [mg/L]	Inhibition relative to control [%]			
		Growth rate based on frond #	Yield based on frond #	Growth rate based on dry weight	Yield based on dry weight
0.41	0.36	-1.2[a]	-3.3	0.1	0.3
1.23	1.14	-1.0	-2.7	0.3	0.8
3.70	3.5	1.6	4.3	5.3	12.3
11.1	11.1	36.1	64.8	52.8	76.9
33.3	34.2	62.7	86.2	80.7	93.2
100	103.7	73.0	91.3	94.9	98.5

a: negative sign indicates stimulation

No morphological effects were observed up to 3.70 mg/L nominal. At 11.1, 33.3 and 100 mg/L about two third of the fronds appeared smaller and one frond was necrotic from day 5. The endpoints as reported by the author are presented below.

B.9.2.1.1-11. Reported endpoints for *Lemna gibba*.

Parameter	Nominal concentrations [mg/L]	Geometric mean concentrations [mg/L]
Growth rate based on frond numbers	EC_{10}: 3.3 95% CL: 1.9 – 4.8 EC_{50}: 25.0 95% CL: 20.3 – 31.1	EC_{10}: 3.2 95% CL: 1.8 – 4.7 EC_{50}: 25.3 95% CL: 20.5 – 31.5
Growth rate based on dry weight	EC_{10}: 3.4 95% CL: 2.5 – 4.3 EC_{50}: 12.0 95% CL: 10.6 – 13.7	EC_{10}: 3.3 95% CL: 2.4 – 4.1 EC_{50}: 12.0 95% CL: 10.5 – 13.7
Yield based on frond numbers	EC_{10}: 3.8 95% CL: 2.4 – 4.8 EC_{50}: 9.2 95% CL: 7.9 – 10.6	EC_{10}: 3.5 95% CL: 2.3 – 4.6 EC_{50}: 9.1 95% CL: 7.8 – 10.6
Yield based on dry weight	EC_{10}: 3.4 95% CL: 2.9 – 3.8 EC_{50}: 7.3 95% CL: 6.8 – 7.8	EC_{10}: 3.2 95% CL: 2.7 – 3.6 EC_{50}: 7.1 95% CL: 6.6 – 7.6

Remarks by RMS

Analytical certificate of the test substance is not submitted. The results an E_rC_{50} of 25.3 mg/L based on frond numbers and 12.0 mg/L based on dry weight and an E_bC_{50} of 9.1 mg/L based on frond numbers and 7.1 mg/L based on dry weight, all based on mean measured concentrations, can be used for risk assessment.

STUDY IIA 8.6/02 (BASF)

Reference/notifier	:	Hoffmann, F. (2011a)	GLP statement	:	yes
Type of study	:	Lemna, growth inhibition	Guideline	:	OECD 221; OPPTS 850.4400 (draft)
Year of execution	:	2011	Acceptability	:	acceptable
Test substance	:	Bentazone-sodium (Reg no 88691). Batch nr.COD-001417, purity 91.9 %, appearance: solid.	Previous evaluation	:	Submitted for renewal (relevant)

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [mg/L]
bentazone-sodium	*Lemna gibba*	static	24 - 25	7.48 – 8.47	7	ErC_{50}	18.6
						EbC_{50}	8.6

Description

Methods. The toxicity of bentazone-sodium to *Lemna gibba* was assessed under static conditions by exposure through the water phase. Nominal test concentrations 0.41, 1.23, 3.70, 11.1, 33.3, and 100 mg/L (corrected for purity), culture medium control. Test medium was 20X AAP medium according to OECD guideline, pH adjusted to 7.5. Test units contained 160 mL test medium, three replicates for the test treatments and six for the control. Two plants with four fronds and one plant with three fronds per replicate (total 11 fronds/replicate). Number of fronds was determined on days 3, 5 and 7 and observation on chlorosis, necrosis, chlorosis, changes in plant size of shape and root growth were made. In addition the biomass based on dry weight was determined at test initiation and at test termination.

Conditions. Temperature controlled incubator at 24 – 25 ºC, continuous light (8300 lux).

Chemical analysis. Samples were taken at the start and at the end, analysis by HPLC-UV (230 nm) after dilution with acetonitrile/water mixture and acidification with sulfuric acid, method recovery 97.7 – 103.9% and 96.9 – 101.6% for fresh and aged fortified samples, respectively. LOQ: 0.1 mg/L.

Calculations and statistics. Mean plant and frond counts were used to calculate the percent inhibition. Numbers of plants and fronds, and dry weight was evaluated by probit analysis using the Chi-square test. Statistical program used was TOXRAT Professional version 2.10.

Results

Measured concentrations ranged from 101.6% to 106.5% of nominal at the start and from 74.8% to 110.3%of nominal at the end. Geometric mean measured concentrations were 0.36, 1.12, 3.5, 11.2, 35.9, and 103.9 mg/L, respectively. Frond numbers in the control showed an 11.2-fold multiplication in seven days. Mean number of fronds after 7 days was 123.5 in control and 134.7, 124.3, 114.7, 55.3, 25.3, and 17.7 at the respective test concentrations. A summary of inhibition percentages is presented below.

Table B.9.2.1.1-12. Inhibition of growth rate and yield of *Lemna gibba* after 7 days exposure to bentazone-sodium.

Nominal concentration [mg/L]	Mean measured concentration [mg/L]	Inhibition relative to control [%]			
		Growth rate based on frond #	Yield based on frond #	Growth rate based on dry weight	Yield based on dry weight
0.41	0.36	-3.6[a]	-9.9	0.0	0.0
1.23	1.12	-0.3	-0.7	0.7	1.8
3.70	3.5	3.1	7.9	5.5	12.9
11.1	11.2	33.3	60.6	41.1	66.7
33.3	35.9	65.6	87.3	63.3	84.5
100	103.9	80.5	94.1	94.5	98.4

a: negative sign indicates stimulation

A dose-response inhibition of growth rate based on frond numbers was found (ranged from -3.6% (stimulation) to 80.5% inhibition). No morphological effects were observed up to 1.23 mg/L nominal. At 3.70 and 11.1 mg/L about one third of the fronds appeared smaller from day 5 on. At 33.3 and 100 mg/L two third of the fronds were smaller from day 5 on and the roots were smaller at test termination.

Table B.9.2.1.1-13. Reported endpoints for *Lemna gibba*.

Parameter	Nominal concentrations [mg/L]	Geometric mean concentrations [mg/L]
Growth rate based on frond numbers	EC_{10}: 3.9 95% CL: 2.7 – 5.1 EC_{50}: 22.2 95% CL: 19.2 – 25.6	EC_{10}: 3.8 95% CL: 2.7 – 4.9 EC_{50}: 23.0 95% CL: 20.0 – 26.4
Growth rate based on dry weight	EC_{10}: 3.6 95% CL: 2.6 – 4.7 EC_{50}: 18.1 95% CL: 15.8 – 20.7	EC_{10}: 3.5 95% CL: 2.5 – 4.6 EC_{50}: 18.6 95% CL: 16.0 – 21.5
Yield based on frond numbers	EC_{10}: 3.4 95% CL: 2.5 – 4.1 EC_{50}: 9.7 95% CL: 8.7 – 10.9	EC_{10}: 3.2 95% CL: 2.3 – 3.9 EC_{50}: 9.8 95% CL: 8.6 – 11.0
Yield based on dry weight	EC_{10}: 2.7 95% CL: 1.9 – 3.4 EC_{50}: 8.7 95% CL: 7.6 – 9.9	EC_{10}: 2.5 95% CL: 1.8 – 3.2 EC_{50}: 8.6 95% CL: 7.5 – 9.9

Remarks by RMS

Analytical certificate of the test substance was not submitted. The results an E_rC_{50} of 23.0 mg/L based on frond numbers and 18.6 mg/L based on dry weight and an E_bC_{50} of 9.8 mg/L based on frond numbers and 8.6 mg/L based on dry weight, all based on mean measured concentrations, can be used for risk assessment.

4.5 Chronic toxicity

4.5.1 Fish early-life stage (FELS) toxicity test

B.9.2.2 Chronic toxicity

B.9.2.2.1 Laboratory testing (IIA 8.2.2; IIA 8.2.4; IIA 8.2.5; IIA 8.3.2; IIA 8.5.2; IIA 8.11)

STUDY IIA 8.2.4/01 (BASF)

Reference/notifier	: Anonymous (2011b)	GLP statement	: yes
Type of study	: fish, early life stage toxicity	Guideline	: US EPA-FIFRA 72-4, EPA-OPPTS 850.1400, and OECD 210 (1992)
Year of execution	: 2011	Acceptability	: acceptable
Test substance	: Bentazone-sodium, BAS 351 H-Na), batch COD-001417, purity 91.9 % appearance yellowish solid. Water solubility up to 120 mg/L.	Previous evaluation	: Submitted for renewal (relevant)

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [mg/L]
bentazone-sodium	*Pimephales promelas*	flow-through	24.7 26.1	7.7 – 8.1	35	NOEC	10

Description

Methods. Early life stages of fathead minnow were exposed to bentazone-sodium under flow-through conditions. The parental fish were hatched and raised at the test facility. Parent fish were acclimated for at least 14 days. Nominal concentrations 10.9 mg test substance/L (10 mg/L corrected for purity) and a control. Dilution with non-chlorinated charcoal filtered drinking water diluted with deionised water, total hardness 95 - 104 $CaCO_3$ mg/L, conductivity 238 - 257 µS. Eggs (< 3.5 h old, blastodisc cleavage stage) from in-house spawning groups of fish were incubated in glass vessels placed in flow-through chambers until hatching. The juveniles were transferred into a larger stainless steel aquarium for the remainder exposure period. Four replicates per concentration, each replicate consisting of 25 fertilised eggs. Flow-through chambers with 1.7 L test solution and flow-rate of 2.25 L/h. Flow-through system for juveniles: 9-L aquarium and a flow rate of 9.0 L/h. Daily observation of egg mortality until hatch. Daily observation of larvae for mortality and signs of toxicity or abnormal behaviour until the end of swim-up and afterwards once a week to the end of the exposure.

Conditions. 25 ºC, 16:8 h L:D (127 -249 lux), slight aeration from day 18 through the end of exposure, due to low oxygen (≥ 64 % saturation). Feeding initiated at the end of the hatch (day 5) with live brine shrimp nauplii and a fine commercial fish diet three times on workdays and twice on non-working days.

Chemical analysis. Water samples were taken at days 0, 9, 16, 23, 30, and 35. Samples were analysed by HPLC with UV detection at 230 nm after dilution with acetonitrile/water mixture and acidified with sulphuric acid. Mean procedural recovery 101%, LOQ 1 mg/L.

Calculations and statistics. Fisher's exact test for treatment comparison with the control for survival. Statistical variability's between replicates with Wilcoxon-test (one-sided). Body weights and length using Student's test (two sided) for comparison of treatment with control.

Results

Mean measured concentration was 9.833 mg/L (98.3% of nominal). On day 9 samples from all replicates were measured, % CV was 0.9%. Hatching in the control was 97% (range 92 – 100%) and started on day 3 and was

completed by day 5. Hatching in the test group was 98% (range 96 – 100%; not significantly different from control). Larvae survival (from hatch until end of swim-up) was between 96 – 100% in the test group (not significant). Start and end of swim up was equal in control and test groups. Juvenile survival in the control was 94% (range 91 – 100%) and in the test group 91% (range 84 – 96%; not significant).. Mean overall survival (day 0 – 35) was 91% (range 84% - 96%) in the control and 88% (range 84 – 92%) in the test group (not significant). No abnormalities were observed in any test and control group. Mean body weight and length were not statistically decreased as compared to control, length was significantly increased as compared to control. As an increase is considered not an adverse effect, this effect was not taken into account for determination of the NOEC. NOEC for survival, body weight and length was set at ≥ 10 mg/L(nominal) and ≥ 9.833 mg/L based on mean measured concentrations of bentazone-sodium.

Remarks by RMS

Water quality criteria within accepted limits. The result NOEC ≥ 10 mg bentazone-sodium/L is used for risk assessment.

4.5.2 Fish short-term toxicity test on embryo and sac-fry stages

Not available.

4.5.3 Aquatic Toxicity – Fish, juvenile growth test

STUDY IIIA 10.2.5/01

Reference/notifier	:	Anonymous (2001)	GLP statement	:	yes
Type of study	:	fish, juvenile growth test	Guideline	:	OECD 215
Year of execution	:	2001	Acceptability	:	Not acceptable
Test substance	:	BENTAZONE 480 g/L SL; purity 480 g/L; batch 0203005; density 1.1916 g/mL	Previous evaluation	:	Submitted for renewal (relevant)

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [mg/L]	Value [mg/L]
BENTAZONE 480 g/L SL	*Oncorhynchus mykiss*	Flow-through		7.6-8.0	28	28-d NOEC juvenile growth	16*	5.91

* nominal

Description

Methods. Toxicity of BENTAZONE 480 g/L SL to survival and growth rainbow trout (Oncorhynchus mykiss) was tested under flow-through conditions. Fish were purchased from a commercial supplier
and were hatched and held in the culture facility of the performing laboratory. Fish were acclimated for
12 days to test conditions, length 51 - 56 mm and mean wet weight 1.56 – 1.64 g at start. Target
concentrations of 6.4, 16, 40, 100 and 250 mg product/L, and a control. Stock solutions were dosed each minute via a computer-controlled system. Dilution with tap water, total hardness 2.1 mmol/L. One replicate with 16 fish each, 30 L water per test vessel, renewal rate 6 L/h. Loading of fish: 0.15-0.18 g fish/L/day. Mortality and sublethal effects were recorded each day. Body weight and length were

determined at the start, after 14 days and at the end of the test (day 28). Fish were sacrificed on day 28. Growth rates were calculated.

Conditions. Temperature 16.2 ± 0.46 °C, 16:8 h L:D, gentle aeration from day 18 as dissolved oxygen levels were approaching 6.0 mg/L (5.7 mg/L at 40 mg/L), fish were fed with commercial trout diet (at 4% of the initial body weight of the fish).

Chemical analysis. Samples were collected at day -2, -1, and at days 0, 7, 14, 21 and 28. Analysis by HPLC- with UV detection at 225 nm after dilution with methanol. Mean procedural recovery was 104% at 2.51 mg as/L and 102% at 99.7 mg as/L, LOQ: 2.51 mg as/L.

Calculations and statistics. Data were analysed with ANOVA. Mortality with Fisher's exact test for comparison of each test group with the control. Dunnett's test (two-sided) and Tukeu HSD test for body weight and length.

Results

Mean measured concentrations were 70 - 93 % of nominal. Mean measured concentrations are given in following table.

Table 9.2.2.1-02. Measured concentrations of bentazone

Concentrations of bentazone (a.i.) and the formulation in samples taken during the study											
Target formulation	Target a.i.	Day 0	Day 7	Day 14	Day 21	Day 28	Mean a.i.	Mean formulation	% Mean of target	SD	% SD of mean
6.4	2.6	2.33	1.20	0.911	1.81	2.88	1.63	4.54	70	0.80	44
16	6.4	6.27	5.10	4.94	6.82	6.42	5.91	14.7	92	0.84	14
40	16	15.6	11.9	11.6	19.2	16.0	14.9	36.9	93	3.14	21
100	40	39.9	27.8	25.6	40.6	35.5	33.9	84.1	85	6.90	20
250	100	96.0	73.8	68.1	103	91.8	86.6	215	87	15.03	17

Mortality: No mortality in control and at 6.4, 16 and 40 mg fonnulation/L, 14 and 15 deaths at 100 and 250 mg fonnulation/L (88 and 94% mortality, respectively), first deaths on days 5-7. 100% mortality in he toxic reference at the highest concentration of 0.46 mg pentachlorophenol/L.

Sublethal effects were: reduced food uptake at 7.5 mg/L, tumbling from day 18 on at 7.5 mg/L (1 to 51 fish), reduced food UP.take was also found at 2.3 mg/L from day 15 on and at 0.75 mg/L from day 20 on.

Mean body weights at day 28 had increased with 212% in the control and with 200, 204 and 186% at 6.4, 16 and 40 mg/L.

Growth rates of the fish were significantly reduced at all concentrations compared with the control (see table below).

Table B.9.2.2.1-02. Mean growth rates

Target formulation mg formulation/L	Mean mg formulation/L	Mean mg as/L	Mean growth rate	
			Interval day 0-14	Interval day 0-28
control	-	-	2.682 a	2.700 a
6.4	4.54	1.83	1.993*bc (26%)	2.456*b (9%)
16	14.7	5.91	2.169*b (19%)	2.558ab (5%)
40	36.9	14.9	1.688*c (37%)	2.188*c (19%)

* significant at 0.05 level with Dunnett's test

a,b,c: Means with the same number are not significantly different (Tukey's Studentised Range (HSD) Test

Fish body length: there were no treatment-related effects on body lengths.

The author calculated a 28-d LC50 for mortality of 68 mg/L with the Finney model. The data however did not fit a probit regression and also failed a logit regression. The 28-day EC50 for juvenile growth was estimated as >36.9 ± 7.8 mg product/L. NOEC for juvenile growth was calculated as 14.7± 2.1 mg product/L.

Remarks by RMS

Water quality parameters within accepted limits. Despite contiuous dosing, the exposure concentration declined up till day 14 (see following table). It is evident that concentrations decrease up to and including day 14. This decrease is strongest at the lowest concentration.

Table B.9.2.2.1-03. Measured concentrations of bentazone expressed as % of nominal

Target formulation	Target formulation in as	% of nominal					% mean of target
		Day 0	Day 7	Day 14	Day 21	Day 28	
6.4	2.6	90	46	35	70	111	70
16	6.4	98	80	77	107	100	92
40	16	98	74	73	120	100	93
100	40	100	70	64	102	89	85
250	100	96	74	68	103	92	87

Thereafter, at days 21 and 28 concentrations were much higher, approximating the initial concentrations measured at day 0 or even higher. This increase of measured concentations at day 21 coincided with a drop of dissolved oxygen at days 15 and 18, but only in the blank control and the three lowest concentrations of 6.4, 16 and 40 mg/L. This drop was not observed at the two highest concentrations. The author argued that the reduction of juvenile growth had recovered after day 14 as the growth reductions at the two lowest concentrations were much lower than during the interval of day 0-14. Thus, the differences in growth rate were assumed to be related to biological variation of the fish rather than to the treatment. The NOEC was set at 14.7 mg product/L based on actual concentrations, equivalent to 16 mg product/L nominal (see Table 9.2.2.1-03). Apparently some unknown facor caused a decrease of growth at all concentrations. In the last 14 days fish were able to make up growth as they had a surplus of inherent growth capacity. At 40 mg as/L the growth however clearly lagged behinded with a reduction of 19%. The growth reduction at the two lower concentrations were 9 and 5% at nominal concentrations of 6.4 and 16 mg product/L, respectively. Taking an effect percentage of 10% as the level of unacceptable effects, the NOEC would be 40 mg formulation/L. At this concentration the effect of bentazone is clearly visible and also significantly different from the control. Significant effects at the two lower concentrations seem to be caused by another factor.

Because of the problems observed inb the study and the inconsistency with other long-term fish studies with the active substance, this study is not considerd ot be reliable.

4.5.4 Chronic toxicity to aquatic invertebrates

STUDY IIIA 10.2.6/01

Reference/notifier :	Migchielsen, M.H.J. (2001)	GLP statement :	yes

Type of study	:	Daphnia, chronic toxicity	Guideline	:	OECD 211 (1998)
Year of execution	:	2001	Acceptability	:	Acceptable Submitted for renewal
Test substance	:	BENTAZONE 480 g/L SL; purity 480 g/L; batch 0203005; density 1.1916 g/mL	Previous evaluation	:	Relevant

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [mg/L]	Value [mg/L]
BENTAZONE 480 g/L SL	*Daphnia magna*	Semi-static	19.4-21.0	7.4-8.6	21	NOEC	80	32[1]

1:corrected for density.

Description

Methods. Chronic toxicity of the metabolite BENTAZONE 480 g/L SL to Daphnia magna was tested under semi-static conditions. Nominal concentrations 25, 45, 80, 145 and 250 mg/L, and a control. Test medium was medium M7, total hardness 250 mgl/L expressed as CaCO3,. Renewal of test concentrations three times a week (Monday, Wednesday and Friday). Ten replicates (neonates <24 h old) per test concentration and 20 replicates in the control, containing one daphnid each. Observations of living, immobile and dead parental daphnids were recorded. Numbers of eggs were recorded every working day. Concerning the F1 generation, appearance of first brood, newborn daphnids and presence of unhatched eggs and immobility were recorded. Length of the parent daphnids was measured at the end of the test.
Conditions. Temperature controlled room at 19.4-21.0 ºC, 16:8 h L:D (964 lux). Daily feeding with green algae. pH 7.4 - 8.3 in fresh media and 7.5 - 8.6 in old media. Dissolved oxygen concentrations 7.3-9.4 mg/L in fresh media and 8.0-9.9 mg/L in old media. Chemical analysis. Samples were taken from 0, 25, 80 and 250 mg/L solutions at the beginning and the end of an interval of 72 hours (days 0 and 3) and of an interval of 48 hours (days 10 and 12 and days 19 and 21). Analysis by HPLC with UV detection at 225 nm after dilution in methanol, LOQ was stated as the lowest concentration of 25 mg as/L. Calculations and statistics. NOEC was determined by ANOVA, followed by Bonferroni-t-test or Tukey test. Reproduction capacity of daphnids was also followed during the total 21-day test period applying the Dynamic Energy Budget theory. For body length ANOVA preceded by Chi-square test for normality was used and Hartley's test for homogeneity of variance.

Results

Mean measured concentrations during the test ranged from 96 - 102% of nominal values. Mortality, total offspring and average body length are summarised in Table B.9.2.2.1-04.

Table B.9.2.2.1-04. Cumulative mortality and production of parental daphnids during the 21-day exposure period

Nominal [mg/L]	Parent Mortality [%]	Reproduction [cumulative offspring/parent]	Average parent body length [mm]	Brood on day 8 [living newborn per vessel]
control	20	187	5.47	7.6
25	10	197	5.53	4.6

45	40	179	5.58	7.4
80	60	204	5.51	9.6
145	30	172*	5.49	8.7
250	10	92#*	5.26#	0.8

The author concluded that mortality of parental daphnids was not treatment related. No reason for the observed mortality could be given. The two highest concentrations significant reduction of reproduction according to the Dynamic Energy Budget theory.

No reduction of parental body lengths was observed at concentrations of 25-145 mg/L compared to the control values. At the highest tested concentration of 250 mg/L a reduction of 4.3% was recorded. This was significant according to Bonferroni-t-test but not according to Tukey's test.

No treatment related effect was observed for the parameter of aborted eggs.

According to the author the overall NOEC was 80 mg product/L.

Remarks by RMS

Water quality parameters within accepted range. Validity criteria were met. The result the 21-d NOEC for reproduction of 80 mg product/L can be used for risk assessment.

STUDY IIIA 10.2.6/01 (BASF)

Reference/notifier	:	Jatzek (1989a)	GLP statement	:	yes
Type of study	:	Daphnia, chronic toxicity	Guideline	:	OECD 211
Year of execution	:	1989	Acceptability	:	acceptable
Test substance	:	BAS 351 32 H (Basagran), batch no. 89-1; content of a.s.: bentazone (BAS 351 H, Reg. No. 519 29, purity: 40.4%): 480 g/L, density: 1.190 g/cm^3	Previous evaluation	:	Original dossier

Substance	Species	Method	Duration [d]	Criterion	Value [mg/L]
Basagran	*Daphnia magna*	Semi-static	21	NOEC	250

Description

Test item: BAS 351 32 H (Basagran), batch no. 89-1; content of a.s.: bentazone (BAS 351 H, Reg. No. 519 29, purity: 40.4%): 480 g/L, density: 1.190 g/cm^3.

Test species: Water flea (Daphnia magna STRAUS), neonates from in-house culture (originally obtained from Institut National de Recherche Chimique Appliquée, France);

Test design: Semi-static system (21 days), 8 test concentrations plus control, ten replicates per treatment and control with one adult daphnid in each test vessel; assessment of parent mortality and reproductive performance three times a week over the 21 day exposure period.

Endpoints: NOEC based on parent mortality and reproduction

Test concentrations: Control (dilution water) and 3.906, 7.81, 15.6, 31.3, 62.5, 125, 250 and 500 mg BAS 351 32 H/L (nominal)

Test conditions: 100 ml test vessels, test volume 50 mL, dilution water: artificial fresh water prepared by adding 15 trace-elements, 10 nutrients and 3 vitamins to deionized water. temperature: 292°K - 294 K; pH: 7.76 - 8.12; oxygen content: 8.16 mg/L - 9.49 mg/L; total hardness: 2.65 - 2.78 mmol/L, conductivity: 620 µS/cm - 650 µS/cm; light intensity: 5 - 6 µEinstein/m2 s1 in the wavelength range of 400 - 750 nm; photoperiod 16 hours light : 8 hours dark; daily feeding with green algae (Scenedesmus subspicatus), no *aeration.*

Analytics: Analytical verification of test item concentrations was conducted.

Statistics: Descriptive statistics; t-Test for comparison of reproduction rates of the treatments groups with the control (carried out only with those values smaller than the reproduction rate of the controls) to determine the EC0 value (α = 0.05, one sided).

Results

Analytical measurements: Analytical verification of the test item concentrations was conducted in all treatments at day 0, 2, 9, 11, 18 and 21. Mean recoveries of BAS 351 32 H in fresh solutions ranged from 97% to 105% of nominal concentrations. During the time interval until renewal of the test solution the mean recoveries were between 96% and 113% of nominal concentrations. The following biological results are based on nominal concentrations.

Biological results: After 21 days of exposure, no parent mortality was observed in the control and at all test item concentrations except at 31.3 mg/L, where 10% of the adult daphnids were found dead after 21 days of exposure. The number of living offspring per parent varied between 39 and 102 in the test item treatments compared to 87 in the control. Day of first brood was day 9 for all test item concentrations and the control. The results are summarized in Table B.9.2.2.1-02.

Concentration [mg/L] (nominal)	Control	3.906	7.81	15.6	31.3	62.5	125	250	500
Parent mortality (21 d) [%]	0	0	0	0	10	0	0	0	0
Av. living offspring/ parent (21 d)	87	90	82	83	81	102	96	88	39
Day of first brood	9	9	9	9	9	9	9	9	9
Endpoints [mg/L] (nominal)									
	based on BAS 351 32 H				based on a.s. (bentazone)				
NOEC = EC0 (21 d) reproduction	250				100.8				
NOEC = LC0 [#] (21 d) mortality	>500				>201.7				

[#] The LC0 is the highest concentration tested with a mean mortality rate lower than or equal to 20%.

Conclusion

Study is acceptable, a NOEC of 250 mg formulation./L, equivalent to 101 mg a.s./L can be used for risk assessment.

4.5.5 Chronic toxicity to algae or aquatic plants

See short-term toxicity

4.5.6 Acute and/or chronic toxicity to other aquatic organisms

Not available.

REFERENCES

Human Health

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Anonymous 1974 (Doc. No. 74/5117)	Anonymous	1974	Metabolism of bentazon in the mouse (musculus) 1974/5117 Unpbulished
Chasseaud L.F. et al. 1972	Chasseaud L.F. et al.	1972a	The metabolic fate of Bentazon in the rat Literature 1972/10076 Xenobiotica, Vol. 2, No. 3, 269-276
Anonymous 2011c (Doc No 2010/1027274)		2011c	Excretion and metabolism of 14C-Bentazone (BAS 351 H, Reg.No. 51929) after oral administration in rats 2010/1027274 Unpublished **Verification of previous metabolite identification with state of the art analytics**
Anonymous 1975 (Doc. No. 75/0083)		1975a	A comparison of the bioavailability and metabolic fate of (14C)-Bentazon free acid and (14C)-Bentazon sodium salt in the rat, after oral administration Huntingdon Research Centre Ltd.; Huntingdon Cambridgeshire PE18 6ES; United Kingdom 1975/0083 unpublished
Anonymous 2011d (Doc No 2011/1197846)		2011d	Amendment No.1 - Excretion and metabolism of 14C-Bentazone (BAS 351 H, Reg.No. 51929) after oral administration in rats 2011/1197846 Unpublished **Verification of previous metabolite identification with state of the art analytics**
Anonymous 1987 (Doc. No. 87/0429)		1987a	The biokinetics and metabolism of 14C-Bentazon in rats 1987/0429 unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Anonymous 2011a (Doc No 2011/1265806)		2011a	14C-BAS 351 H (Bentazone) - Study on the effect of Probenecid on plasmakinetics in rats 2011/1265806 Unpublished **Provide plasma kinetics over time in currently used rat strain**
Anonymous 1971 (Doc. No. 71/0069)		1971a	The metabolism of Bentazon in rats 1971/0069 unpublished
Anonymous 2011b (Doc No. 2011/1262233)		2011b	14C-BAS 351 H (Bentazone) - Study on the plasmakinetics in rats 2011/1262233 Unpublished **Provide plasma kinetics over time in currently used rat strain**
Anonymous 1974 (Doc. No. 74/5116)		1974a	Metabolism and balance study of 14C-BAS 351-H in rabbits 1974/5116 unpublished
Anonymous 1974 (Doc. No. 74/9000)		1974a	Investigations of rabbit urine and feces after oral administration of 14C-Bentazon 1974/9000 unpublished
Anonymous 1986 (Doc. No. 86/090)		1986a	Investigation of urinary metabolites of Bentazon in the rat 1986/090 unpublished
Anonymous 1985 (Doc. No. 85/299)		1986b	Dermal absorption of 14C-Bentazon in rats 1985/299 unpublished
Anonymous 1972 (Doc. No. 72/051)		1972	Bericht über die Prüfung der akuten oralen Toxiczität von Bentazon tech. an der Ratte. 72/051 Unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Anonymous 1983 (Doc. No. 83/114)		1983a	Report on the study of the acute oral toxicity in rats of Reg.No. 51 929 1983/114 unpublished
Anonymous 1973 (Doc. No. 73/022)		1973a	Acute oral toxicity of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide to the rat 1973/022 unpublished
Anonymous 1973 (Doc. No. 73/023)		1973b	Acute oral toxicity of the sodium salt of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide to the rat 1973/023 unpublished
Anonymous 1978 (Doc. No. 78/053)		1978a	Acute oral, subcutaneous and intraperitoneal toxicity studies of Bentazon-acid in the rat 1978/053 unpublished
Anonymous 1983 (Doc. No. 83/113)		1983b	Report on the study of acute oral toxicity in the rat of Reg.No. 51 929 1983/113 unpublished
Anonymous 1969 (Doc. No. 69/0013)		1969a	Acute oral toxicity of Thianon in rats 1969/0013 unpublished
Anonymous 1991 (Doc. No. 91/10147)		1991a	Report on the study of the acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinon-(4)-2,2-dioxide (= Bentazon) in guinea pigs 1991/10147 unpublished
Anonymous 1974 (Doc. No. 74/035)		1974a	Acute oral toxicity of the sodium salt of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide (Bentazon) to the guinea pig 1974/035 unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Anonymous 1970 (Doc. No. 70/016)		1970a	Acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)-2,2-dioxide to cats 1970/016 unpublished
Anonymous 1970 (Doc. No. 70/017)		1970b	Acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)-2,2-dioxide in dogs 1970/017 unpublished
Anonymous 1969 (Doc. No. 69/005)		1969a	Acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)-2,2-dioxide (technical grade) on rabbits 1969/005 unpublished
Neuschl J and Kacmar P, 1993	Neuschl J and Kacmar P	1993a	Acute oral toxicity of the herbicide Bentazone developed in Czechoslovakia with regard to pheasants and rabbits and the clinical symptomatology of the toxicity the clinical symptomatology of the toxicity (Slovak) Literature 1993/11411 Vet. Med. - Czech, 38 (2), 115-121
Anonymous 1986 (Doc. No. 86/195)		1986a	Report on the maximization test for the sensitizing potential of Reg.No. 51 929 - Bentazon in guinea pigs 1986/195 unpublished
Anonymous 1986 (Doc. No. 86/221)		1986a	Report on the Open Epicutaneous Test (OET) for the sensitizing potential of Bentazon-Na 600 g/L in the guinea pig 1986/221 unpublished
Klecak G, 1977	Klecak G.	1977a	Identification of contact allergens: Predictive tests in animals Literature 1977/10334 Dermatotoxicology and Pharmacology, Advances in Modern Toxicology, Vol. 4, 305-339

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Anonymous 1981 (Doc. No. 81/10240)		1981a	One month toxicity tests for Bentazon in rats (tests to determine the dosage levels for 24-month toxicity tests) 1981/10240 unpublished
Anonymous (1981) (Doc. No. 81/10239)		1981a	Thirty-day oral toxicity study of Bentazon in mice 1981/10239 unpublished
Anonymous 1970 (Doc. No. 70/008)		1970a	90-day feeding trial on rats with 3-isopropyl-2,1,3-benzothiadiazinone-(4)2,2-dioxide 1970/008 unpublished
Anonymous 1987 (Doc. No. 87/0173)		1987a	13-week oral toxicity (feeding) study with Bentazon technical (ZNT No. 86/48) in the rat 1987/0173 unpublished
Anonymous 2011 (Doc No. 2011/1173365)		2011a	Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) and Reg.No. 51929 (Bentazone-acid, BAS 351 H) – A comparative repeated dose 90-day oral toxicity study in Wistar rats – Administration via the diet. 2011/1173365 Unpublished
Anonymous 2012 (Doc No. 2012/1009658)		2012a	Amendment No. 1: Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) and Reg.No. 51929 (Bentazone-acid, BAS 351 H) – A comparative repeated dose 90-day oral toxicity study in Wistar rats – Administration via the diet 2012/1009658 Unpublished
Anonymous 1970 (Doc. No. 70/009)		1970a	13-week toxicity of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide (hereafter referred to as XIX/410) to beagles when administered with the food 1970/009 unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Anonymous 1973a (Doc. No. 73/0057)		1973a	13 weeks oral toxicity study in beagle dogs with 3-isopropyl-2,1,3-benzothiadiazinon-(4)-2,2-dioxid (here called Bentazon) 1973/0057 unpublished
Anonymous 1972a (Doc. No. 72/0061)		1972a	13 weeks oral toxicity study in beagle dogs with 3-isopropyl-2,1,3-benzothiadiazinon-(4)-2,2-dioxid (here called Bentazon) 1972/0061 unpublished
Anonymous 1989 (Doc. No. 89/0049)		1989a	52-week oral toxicity (feeding) study with Bentazon technical (ZST No.: 86/48) in the dog 1989/0049 unpublished
Anonymous 1989 (Doc. No. 89/0153)		1989a	1st amendment to report: 52-week oral toxicity (feeding) study with Bentazon technical (ZST No.: 86/48) in the dog 1989/0153 unpublished
Anonymous 1993 (Doc. No. 93/10760)		1993a	Study of the dermal toxicity of Reg.No. 51 929 in white rabbits - Application to the intact skin over 3 weeks 1993/10760 unpublished
Anonymous 1988 (Doc. No. 88/0350)		1988a	Study on the dermal toxicity of Reg.No. 51 929 / Bentazon in rabbits - Application to the intact skin for 3 weeks (21 applications) 1988/0350 unpublished
Anonymous 1971 (Doc. No. 71/005)		1971b	21-day toxicity of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide to NZW rabbits on local application 1971/005 unpublished
Anonymous (1974) (Doc. No. 74/004)		1974a	Two year chronic oral toxicity study of BAS 351-H in rats 1974/004 unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Anonymous 1985 (Doc. No. 85/433)		1984a	Studies on the 24-month chronic toxicity of Bentazon in rats 1985/433 unpublished
Anonymous 1985 (Doc No 85/440)		1985a	Review of the studies on the 24 months oral chronic toxicity and potential carcinogenicity of Bentazon in rats 1985/440 unpublished
Anonymous 1986 (Doc No 86/0438)		1986a	Supplemental report of the studies on the 24-month oral chronic toxicity and potential carcinogenicity of Bentazon in rats 1986/0438 unpublished
Anonymous 1988 (Doc No 88/0483)		1988a	Additional histopathological investigation into the salivary and mammary glands taken from the long term feeding study in mice with Bentazon 1988/0483 unpublished
Anonymous 1974 (Doc. No. 74/041)		1974b	18 month chronic oral toxicity study of BAS 351-H in mice 1974/041 unpublished
Anonymous 1978 (Doc. No. 78/034)		1978a	Tumorigenicity of Bentazone acid to mice in long term dietary administration 1978/034 unpublished
Anonymous 1985 (Doc. No. 85/432)		1984b	Studies on the 24-month chronic toxicity of Bentazon Reg.No. 51 929 (ZNT No. 81/273) in mice 1985/432 unpublished
AnonymousButler WH, 1985 (Doc No 85/0442);		1985b	Review of the studies on the 24 months oral chronic toxicity and potential carcinogenicity of Bentazon Reg.No. 51 929 (ZNT No. 81/273) in mice 1985/0442 unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Anonymous 1985 (Doc No 85/431)		1985a	Supplemental report of the studies on the 24-month chronic toxicity of Bentazon Reg.No. 51 929 (ZNT No. 81/273) in mice 1985/431 unpublished
Anonymous 1987 (Doc No 87/0139)		1987a	Review of hepatic and pulmonary tissues of 24-month chronic oral toxicity study of Bentazon Reg.No. 51 929 in mice 1987/0139 unpublished
Anonymous 1989 (Doc No 1989/10485)		1989a	Bentazon-review of additional data concerning the two-year chronic toxicity/oncogenicity studies in rats and mice 1989/10485 unpublished
Anonymous 1989 (Doc. No. 89/0068),		1989a	Two-generation reproduction study with Bentazon technical (ZST-No. 86/48) in the rat 1989/0068 unpublished
Anonymous 2011 (Doc. No. 2011/1248852)		2011a	Bentazone (BAS 351 H): Reassessment of the maternal and developmental toxicity in the 2-generation study 2011/1248852 No, not subject to GLP regulations unpublished **Historical control data for reassessment**
Anonymous 2011 (Doc No 2011/1145234)		2011a	8 Two-generation studies 1985-1990 in the Han Wistar rat (KFM:WIST) performed at RCC Ltd. 2011/1145234 No, not subject to GLP regulations Unpublished **Relevant open literature**

reference no.	**Author(s)**	**Date Year / Month / Day**	**Title** **Source** **DocID** **Published or not**
Anonymous 2009 (Doc No 2011/1262290)		2009a	Influence of litter size on the postnatal growth of rat pups: Is there a rationale for litter-size standardization in toxicity studies? 2011/1262290 No, not subject to GLP regulations Environmental Research 109 (2009) 1021-1027 **Relevant open literature**
Anonymous 1973 (Doc. No. 73/010)		1973a	Chronic oral toxicity of Bentazon in a reproduction study covering three generations of Sprague Dawley rats 1973/010 unpublished
Anonymous 1991 (Agrichem file no. R 22)		1991	Dose range-finding embryotoxicity study (including teratogenicity) with bentazon in the rat RCC, 266861 unpublished
Anonymous 1991 (Agrichem file no. R 463)		1991	Dose range-finding embryotoxicity study (including teratogenicity) with bentazon in the rat RCC, 266872 prat I and part II unpublished
Garagna et al. 2005	Garagna et al.	2005	Effects of a low dose of bentazon on spermatogenesis of mice exposed during foetal, postanatal and adult life Toxicology 2005; 212: 165-174 published
Orton et al. 2009	Orton et al.	2009	Endocrine disrupting effects of herbicides and pentachlorophenol in vitro and in vivo evidence. Envrion. Sci. Technol. 2009; 43: 2144-2150 published
Roncaglioni et al. 2008	Roncaglioni et al.	2008	Binary classification models for endocrine disrupter effects mediated through the estrogen receptor. SAR and QSAR in Environmental Research 2008; 19: 697-733 published

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Kojima 2004	Kojima	2004	Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cels Environmental Health Perspectives 2004; 5: 524-531 published
Bauer 2002	Bauer	2002	Development of an immuno-immoblilized androgen receptor assay (IRA) and its application for the characterization of the receptor binding affinity of different pesticides Chemosphere 2002; 46: 1107-1115 published
Bitsch 2002	Bitsch	2002	In vitro screening of the estrogenic activity of active components in pesticides Z. Umweltchem. Ökotox 2002; 14: 76-84 published
Anonymous 1986 (Doc. No. 86/421)		1986a	Embryotoxicity (including teratogenicity) study with Bentazon technical in the rat 1986/421 unpublished
Anonymous 1982 (Doc. No. 84/066)		1982a	Teratogenicity study of Bentazon, Reg.No. 51 929 (ZNT No. 81/273) in rats by dietary administration 1984/066 unpublished
Anonymous 1978 (Doc. No. 78/039)		1978a	Investigation to determine the prenatal toxicity of 3-isopropyl-2,1,3-benzothiadiazin-4-one-2,2-dioxide on rats 1978/039 unpublished
El-Mahdi MM and Lofti MM (1988)	El-Mahdi MM and Lofti MM	1988a	Teratological effects of pesticide (Basagran) on embryo of albino rat Literature 1988/10538 Arch. exper. Vet. med., 42, 2, 261-266

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Anonymous 1971 (Doc. No. 71/0041)		1971	Bericht über die Prüfung von 3-isopyropyl-2,1,3-benzo-thiadiazinon-(4)-2,2-dioxid (= bentazon) auf etwaige teratogene Wirkung an der Ratte bei peroraler Applikation 71/0041
Anonymous 1984 (Doc. No. 84/048)		1978b	Study to determine the prenatal toxicity of 3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-on-2,2-dioxide in rabbits 1984/048 Yes, study was conducted prior to the implementation of GLP certificates unpublished
		1984a	Amendment to report: Study to determine the prenatal toxicity of 3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-on-2,2-dioxide in rabbits 1984/288 unpublished
Anonymous 1987 (Doc. No. 87/058)		1987b	Embryotoxicity (including teratogenicity) study with Bentazon technical in the rabbit RCC – Research & Consulting Company AG; Itingen; Switzerland 1987/058 unpublished

Environment

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Anonymous 1983 STUDY IIA, 8.2.1.2/02	Anonymous	1983	Report on the study of the acute toxicity - Bentazon-Na - Carp (Cyprinus carpio L.) BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1983/10048 Yes unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Anonymous 1986a. STUDY IIA, 8.2.1.2/01	Anonymous	1986	Report on the study of the acute toxicity - Reg.No. 51 929 (Bentazon) - Bluegill (Lepomis macrochirus RAF.) BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1986/9005 yes unpublished
Anonymous 1987a. STUDY IIA, 8.2.1.1/01	Anonymous	1987a.	Report on the study of the acute toxicity - Reg.No. 51 929 - Rainbow trout (Salmo gairdneri RICH.) BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1987/0428 Yes unpublished
Anonymous 2001 Study IIIA 10.2.5/01	Anonymous	2001	not listed in RAR reference list
Bergström L. et al. 1994b Study IIA 7.4.1/01	Bergström L. et al.	1994b	Pesticide leaching data to validate simulation models for registration purposes <none>; <none>; <none> 1994/10464 No, not subject to GLP regulations J. Environ. Sci. Health, A29(6), 1073-1104
Bias 1986a Study IIA 8.3.1.1/05	Bias	1986a	not listed in RAR reference list
Bieber, 1994 Study IIA 7.8.3	Bieber W.-D.	1994a	Degradation of the test substance Bentazon in aerobic aquatic environment NATEC - Institut fuer naturwissenschaftlich-technische Dienste GmbH; Hamburg; Germany Fed.Rep. 1994/11026 Yes unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Boivin A. 2003b thesis Study IIA 7.4.1/04	Boivin A.	2003b	Disponibilite spatio-temporelle et transfert des pesticides dans le sol <none>; <none>; <none> 2003/1032519 No, not subject to GLP regulations These: Institut National Polytechnique de Lorraine, Ecole Nationale Superieure d Agronomie et des Industries Alimentaires
Boivin A. 2004b Study IIA 7.4.1/07	Boivin A.	2004b	Time effect on Bentazone sorption and degradation in soil <none>; <none>; <none> 2004/1015218 No, not subject to GLP regulations Pest Management Science 60: 809-814 (online: 2004) DOI: 10.1002/ps.889
Boivin A. et al. 2005b Study IIA 7.4.1/05;	Boivin A. et al.	2005b	A comparison of five pesticides adsorption and desorption processes in thirteen contrasting field soils <none>; <none>; <none> 2005/1041040 No, not subject to GLP regulations Chemosphere 61 (2005)
Boivin A. et al. 2005c Study IIA 7.4.1/06	Boivin A. et al	2005c	entazone adsorption and desorption on agricultural soils <none>; <none>; <none> 2005/1041502 No, not subject to GLP regulations Agron. Sustain. Dev. 25 (2005) 309-315
Budde, E., 2014a Study IIA 7.1.1/00	Budde, E.	2014a	not listed in RAR reference list
Class T., 2005b Study IIA 7.2.3/01	Class T	2005b	Aerobic soil degradation of N-Methyl-Bentazone in three standard soils at 20°C PTRL Europe GmbH; Ulm; Germany Fed.Rep. 2005/1026922 Yes Unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Dawson, I., R. Lynn & G.Y. McCorquodale 2003 Study IIA 7.6/03	Dawson, I., R. Lynn & G.Y. McCorquodale	2003	[^{14}C]-Bentazone. Artificial sunlight degradation of [^{14}C]-bentazone in buffered aqueous solution. Inveresk report no. 21825, Tranent, Scotland. GLP Not published
De Vette, H.Q.M, Nachtegaal R.M.A., van Es C. 2002 Study IIA 7.1.3/02	De Vette, H.Q.M, Nachtegaal R.M.A., van Es C.	2002	not listed in RAR reference list
De Vries 1996 Study IIA 7.8.3/02	De Vries, R.	1996	Degradation of Bentazon in aerobic aquatic environment, NOTOX, 143898 GLP Not published
Dohmen 1990a Study IIA, 8.4/03	Dohmen	1990a	Effect of Bentazon on the growth of the green alga Ankistrodesmus bibraianus BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1990/0167 Yes unpublished
Dohmen 1990b Study IIA, 8.4/04	Dohmen	1990b	Effect of Bentazon (DEA-salt) on the growth of the green alga Ankistrodesmus bibraianus BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1990/0166 Yes unpublished
Drescher and Otto, 1972 Study II A 7.1.1	Drescher and Otto	1972	Degradation of Bentazon (BAS 351-H) in soil - 2nd report BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 72/10064 No, studies were conducted prior to the implementation of GLP but are scientifically valid unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Drescher and Otto 1973a Study II A 7.1.1	Drescher and Otto	1973a	Degradation of Bentazon (BAS 351-H) in soil - 2nd report BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1973/0031 No, studies were conducted prior to the implementation of GLP but are scientifically valid unpublished
Ebert, D. 2010b Study II A 7.3.1/03	Ebert, D.	2010b	Anaerobic soil metabolism of Bentazon (BAS 351 H) BASF SE; Limburgerhof; Germany Fed.Rep. 2010/1005602 Yes Unpublished
Ebert, D. 2000a Study II A 7.1.1	Ebert, D.	2000a	Degradation of Bentazon (BAS 351 H) in lysimeter soil (Borstel, northern Germany) BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2000/1000142 Yes unpublished
Eswein and Panek 1986a Study IIA 2.9	Eswein, R.P. and Panek, E.J.	1986a	Hydrolysis of Bentazon in pH 5, 7, and 9 solutions at 25°C. BASF Reg. Doc. #86/5018. WAS95-00101. No Unpublished
Evans 1994 Study II A 7.8.3	Evans, J. R.	1994	Basagran herbicide dissipation in a rice paddy 1992 study: Final report BASF Corp. Agricultural Products Center; Research Triangle Park NC; United States of America 1994/5151 Yes unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Gerhardt and Hamm, 1987 Study II A 7.8.3	Gerhardt R., Hamm R.T.	1987a	The aerobic aquatic metabolism of Bentazon (BAS 351 H) BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1987/0417 No, studies were conducted prior to the implementation of GLP but are scientifically valid unpublished
Gerhardt and Hamm, 1987 Study II A 7.8.3	Gerhardt R., Hamm R.T.	1987b	The anaerobic aquatic metabolism of Bentazon (BAS 351 H) BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1987/0416 No, studies were conducted prior to the implementation of GLP but are scientifically valid unpublished
Graves, W.C. and Smith G.J. 1991a Study IIA 8.11.1/01	Graves, W.C. and Smith G.J.	1991a	Bentazon: A 96-hour flow-through acute toxicity test with the saltwater mysid (Mysidopsis bahia) Wildlife International Ltd.; Easton MD; United States of America 1991/5192 Yes Unpublished **Requirement for US registrations**
Graves, W.C. and Smith, G.J. 1992a Study IIA 8.11.1/03	Graves, W.C. and Smith G.J.	1992a	Bentazon: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica) Wildlife International Ltd.; Easton MD; United States of America 1992/5017 Yes unpublished Requirement for US registrations
Hassink J. 2012a Study II A 7.1.3/01	Hassink J.	2012a	Soil photolysis of 14C-Bentazone BASF SE; Limburgerhof; Germany Fed.Rep. 2011/1276919 Yes Unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Hassink J. 2012b Study II A 7.1.3/01	Hassink J.	2012b	Report Amendment No. 1 - Soil photolysis of 14C-Bentazone BASF SE; Limburgerhof; Germany Fed.Rep. 2012/1023466 Yes unpublished
Hoek, van der E & J.F. Kreuk 1987 Study IIA 7.7/01	Hoek van der E., Kreuk de J.F.	1987	Biodegradability of Bentazone according to OECD 301 B (Modified sturm-test), TNO, R 87/277 GLP Not published
Hoffmann, F. 2011a Study IIA 8.6/02	Hoffmann, F.	2011a	Effect of Bentazone-Na (Reg.No. 88691) on the growth of Lemna gibba BASF SE; Limburgerhof; Germany Fed.Rep. 2011/1102366 Yes Unpublished To show ecotoxicological equivalence of registered acid and manufactured sodium product
Hoffmann, F. 2011b Study IIA 8.6/01	Hoffmann, F.	2011b	Effect of Bentazone (Reg.No. 51929) on the growth of Lemna gibba BASF SE; Limburgerhof; Germany Fed.Rep. 2011/1102365 Yes unpublished To show ecotoxicological equivalence of registered acid and manufactured sodium product
Housari F.A. et al. 2010a Study IIA, 7.6/02	Housari F.A. et al.	2010a	Factors responsible for rapid dissipation of acidic herbicides in the coastal lagoons of the Camargue (Rhone River Delta, France) <none>; <none>; <none> 2011/1276854 No, not subject to GLP regulations Science of the Total Environment 409 (2011) 582-587

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Huber, 1994 Study II A 7.1.1	Huber R., Otto S.	1994	Environmental behavior of Bentazon herbicide Literature 1994/10340 new No, not subject to GLP regulations Reviews of Environmental contamination and Toxicology, Vol. 137, 111-134
Hughes 1991a Study IIA, 8.6/06	Hughes J.S., Alexander M.M.	1991a	The toxicity of Bentazon (BAS 351 H Tech. a.i.) to Lemna gibba G3 Malcolm Pirnie Inc.; Tarrytown NY; United States of America 1991/5195 Yes unpublished
Jatzek 1989a Study IIIA 10.2.6/01	Jatzek, H.J.	1989a	Determination of the longterm effects of Basagran BAS 351 32 H charge 89-1 on the parthenogenetic reproduction rate of the waterflea Daphnia magna STRAUS BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1989/10083 Yes unpublished
Jatzek, H.J. 2003b Study IIA 8.3.1.1/01	Jatzek, H.J.	2003b	BAS 351 H (Bentazone) - Determination of the acute effect on the swimming ability of the water flea Daphnia magna STRAUS BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2003/1004524 Yes Unpublished To close data gap

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Jatzek, H.-J. 2003b Study IIA 8.4/01	Jatzek, H.J.	2003b	BAS 351 H (Bentazone) - Determination of the inhibitory effect on the cell multiplication of unicellular green algae BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 2003/1012046 Yes Unpublished To close data gap
Keller W. 1995b Study IIA, 7.4.1/02	Keller W.	1995(b)	Adsorption study of Bentazon in a Vredepeel soil from the Netherlands BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1995/10689 No Unpublished
Keller, 1986a Study I A 7.4.1	Keller, W.	1986a	Soil adsorption/desorption study of Bentazon BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1986/0457 No, studies were conducted prior to the implementation of GLP but are scientifically valid unpublished
Keller, 1987 BOD95-00264	Keller, E.	1987	Keller, E. (1987). The aerobic soil metabolism of BAS 351 H (Bentazone). BASF DocID 1987/0415.
Keller, 1988 BOD96-124	Keller, W.	1988	Keller, W. (1988). Degradation behavior of Bentazone in soil. BASF DocID 1988/10121.
Larsbo et al 2009a Study IIA 7.4.1/08	Larsbo M. et al.	2009a	Herbicide sorption, degradation, and leaching in three Swedish soils under long-term conventional and reduced tillage <none>; <none>; <none> 2009/1127645 No, not subject to GLP regulations Soil and Tillage Research 105 (2009) 200-208

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Leistra 2001 Study IIA 7.2.1/07	Leistra M. et al.	2001b	Rate of Bentazone transformation in four layers of a humic sandy soil profile with fluctuating water table <none>; <none>; <none> 2001/1021063 No, not subject to GLP regulations Pest Management Science 57, 1023-1032
Li K.-B. et al 2008b Study IIA, 7.2.1/03	Li K.-B. et al.	2008b	Degradation of herbicides Atrazine and Bentazone applied alone and in combination in soils <none>; <none>; <none> 2008/1098933 No, not subject to GLP regulations Pedosphere 18(2), 2008
Matejek B., 2012a Study II A 7.8.3/01	Matejek B.	2012a	Kinetic evaluation of a water-sediment study with BAS 351 H - Bentazon according to FOCUS degradation kinetics BASF SE; Limburgerhof; Germany Fed.Rep. 2011/1285046 No, not subject to GLP regulations Unpublished
Migchielsen, M.H.J. 2001 Study IIIA 10.2.6/01	Migchielsen, M.H.J.	2001	Acute toxicity study in Daphnia Magna with Bentazone 480 g/l SL (static) Notox project no. 318509 GLP Not Published
Philips, M & I.Dawson, (2003) Study IIA, 7.6/04	Phillips, M. & I.M. Dawson,	2003	Isolation and identification of a degradation product generated during artificial sunlight of [^{14}C]-bentazone in buffered aqueous solution. Inveresk report no. 22271, Tranent, Scotland. GLP Not published

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Redeker, 1978, Study II A 7.4.1	Redeker J.	1978a	Determination of the constants of the adsorption isotherm of Bentazon in a soil/water system BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1978/10129 No, studies were conducted prior to the implementation of GLP but are scientifically valid unpublished
Rodriguez Cruz M.S. et al 2008b Study IIA, 7.2.1/04	Rodriguez Cruz M.S. et al	2008b	Study of the spatial variation of the biodegradation rate of the herbicide Bentazone with soil depth using contrasting incubation methods <none>; <none>; <none> 2008/1098934 No, not subject to GLP regulations Chemosphere 73 (2008)
Ross et al,1989, WAS95-00113	Ross et al	1989	not listed in RAR reference list
Seher A. 1999(b) Study IIA, 7.4.1/03	Seher A.	1999b	Adsorption study of 51929 (BAS 351 H) on lysimeter soils BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1999/10685 Yes Unpublished
Singh M., 2011(a) Study IIA, 7.6/01	Singh M.	2011a	Aqueous photolysis of 14C-BAS 351 H BASF Agricultural Research Center; Research Triangle Park NC; United States of America 2011/7002318 Yes unpublished
Smelt J.H. 2003b Study IIA, 7.2.1/05	Smelt J.H.	2003b	Laboratory studies on the degradation rates of Bentazone in Dutch soil profiles BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2002/1011918 Yes Unpublished

reference no.	Author(s)	Date Year / Month / Day	Title Source DocID Published or not
Staudenmaier H., Kuhnke G. 2010b Study II A 7.1.1/01	Staudenmaier H., Kuhnke G.	2010b	Aerobic soil metabolism of ^{14}C-Bentazone BASF SE; Limburgerhof; Germany Fed.Rep. 2010/1057318 Yes Unpublished
Timme and Frehse, 1980 BOD96-000138	Timme and Frehse	1980	not listed in RAR reference list
Timme et al., 1986, BOD96-00139	Timme et al.,	1986	not listed in RAR reference list
Tornisielo A., Sacchi R.R. 2011b Study II A 7.2.1/01	Tornisielo A., Sacchi R.R.	2011b	Rate of degradation of BAS 351 H in European soils under aerobic conditions BASF SA; Guaratingueta; Brazil 2011/1000621 Yes Unpublished
Tornisielo A., Vasques A.C. 2011b Study IIA 7.4.2/01	Tornisielo A., Vasques A.C.	2011b	Adsorption behaviour of BH 351-N-Me (metabolite of BAS 351 H, Bentazone) on different European soils BASF SA; Guaratingueta; Brazil 2011/1021360 Yes Unpublished
Traub, M., 2012 Study IIA 7.2.3/02	Traub, M.,	2012	N-methyl bentazone: aerobic degradation three European soils Eurofins, Germany Study report GLP Not published
Van de Veen J.R. 2003b Study IIA 7.2.1/06	Van de Veen J.R.	2003b	not listed in RAR reference list
Verhaar. H.J.M., 2012 (statement) Study IIA, 7.2.1/02 Study IIA 7.2.3/03	Verhaar. H.J.M.,	2012	Bentazone. Recalculation of soil degradation half lives according to FOCUS Kinetics. ENVIRON, Den Dolder, the Netherlands. Report no. AC-AIR3-20120011 GLP not applicable Not published

reference no.	**Author(s)**	**Date Year / Month / Day**	**Title** **Source** **DocID** **Published or not**
Völkel W. 2001 Study IIA, 7.4.1/09	Völkel W.	2001	Degradation of ^{14}C-Bentazone in one soil incubated under aerobic conditions, RCC, 773875 GLP Not published
Vonk J.W. & van den Hoven A.W. 1987 Study IIA, 7.4.1/10	Vonk J.W. & van den Hoven A.W.	1987	Adsorption of Bentazone to ditch bottom sediment, TNO, R 87/315 Non GLP Not published
Wood, 1986 Study IIA 7.1.3 BOD95-00265	Wood N.F.	1986a	Photolysis of Bentazon on soil BASF Corp.; Parsippany NJ; United States of America 1986/5017 No unpublished