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

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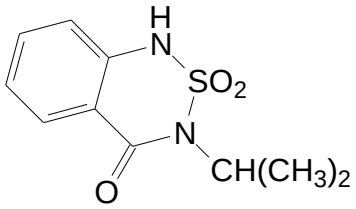
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	3-isopropyl-(1H)-2,1,3-benzothiadiazin-4-(3H)-one-2,2-dioxide
Other names (usual name, trade name, abbreviation)	Bentazone, Bendioxide, 3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide;
ISO common name (if available and appropriate)	Bentazone
EC number (if available and appropriate)	246-585-8
EC name (if available and appropriate)	Bentazone
CAS number (if available)	25057-89-0
Other identity code (if available)	CIPAC No. 366
Molecular formula	$C_{10}H_{12}N_2O_3S$
Structural formula	
SMILES notation (if available)	O=C(N(S(=O)(=O)Nc1cccc2)C(C)C)c12
Molecular weight or molecular weight range	240.3 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	The minimum purity of 960 g/kg related to the theoretically dry active ingredient. Typically bentazone is handled as technical concentrate with ca. 700 g/L bentazone sodium salt (corresponding to 640 g/L bentazone) in water. 960 ± 25 g/kg as given by FAO specification FAO Specification (including year of publication): AGP: CP/ 307, (1994)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Bentazone EC number: 246-585-8	≥96% w/w	Acute Tox. 4* (H302); Eye Irrit. 2 (H319); Skin Sens. 1 (H317); Aquatic Chronic 3 (H412)	Acute Tox. 4 (H302); Eye Irrit. 2 (H319); Skin Sens. 1 (H317); Aquatic Chronic 4 (H412)

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
N/A				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
N/A					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Batch no.	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
Bentazone	COD-001416	100%	N/A		Anonymous 2011 (Doc No: 2011/1173365)
Bentazone	N 187	97.8%	N/A		Anonymous 1989 (Doc. No. 89/0068) Anonymous 1986 (Doc. No. 86/421) Anonymous 1987 (Doc. No. 87/058) Anonymous 1987 (Doc. No. 87/0173) Anonymous 1989 (Doc. No. 89/0049)
Bentazone	N 194	97.64%	N/A		Anonymous 1993 (Doc. No. 93/10760)
Bentazone	270 778	94.6%	N/A		Anonymous (1978) (Doc. No. 78/053)
Bentazone	MS 2 F 22	94.0%	N/A		Anonymous 1986 (Doc. No. 86/195)
Bentazone	N 169	93.9%	N/A		Anonymous 1985 (Doc. No. 85/433) Anonymous 1985 (Doc. No. 85/432) Anonymous 1982 (Doc. No. 84/066)
Bentazone	N/A	93.9%	N/A		Anonymous 1983 (Doc No. 83/114) Anonymous 1981 (Doc. No. 81/10240)
Bentazone	N/A	92.5%	N/A		Anonymous 1978 (Doc. No. 78/039) Anonymous 1984 (Doc. No. 84/048)
Bentazone	p. 195.75	N/A	N/A		Anonymous 1978 (Doc. No. 78/034)
Bentazone	N/A	N/A	N/A		Anonymous 1969 (Doc. No. 69/0013) Neuschl J and Kacmar P, 1993

Identification of test substance	Batch no.	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
					Anonymous 1970 (Doc. No. 70/017) Anonymous 1970 (Doc. No. 70/009) Anonymous 1971 (Doc. No. 71/005) Anonymous 1983 (Doc. No. 83/113) Anonymous 1973 (Doc. No. 73/022) Anonymous 1972 (Doc. No. 72/051) Anonymous 1969 (Doc. No. 69/005) Anonymous 1991 (Doc. No. 91/10147) Anonymous 1970 (Doc. No. 70/016) Anonymous 1974 (Doc. No. 74/004) Anonymous 1974 (Doc. No. 74/041) Anonymous 1973 (Doc. No. 73/010) Anonymous 1971 (Doc. No. 71/0041) Anonymous, 1981 (Doc. No. 81/10239) Anonymous 1970 (Doc. No. 70/008)
Bentazone sodium	COD-001417	91.9%	N/A		Anonymous 2012 (Doc No. 2012/1009658)
Bentazone sodium	B016	N/A	N/A		Anonymous 1991 (Agrichem file no. R 463) Anonymous 1991 (Agrichem file no. R 22)
Bentazone sodium	N/A	N/A	N/A		Anonymous 1973 (Doc. No. 73/023) Anonymous 1974 (Doc. No. 74/035)
Bentazone sodium salt formulation (600 g/L)	WH 4976	N/A	N/A		Anonymous 1986 (Doc. No. 86/221)
Basagran (a bentazone-containing formulation)	N/A	N/A	N/A		El-Mahdi MM and Lofti MM 1988

A part of the available studies was performed with the sodium salt of bentazone. The (eco)toxicological results with sodium bentazone are considered relevant for bentazone because in solution and therefore also in biological systems both substances will dissociate and form the same anion. In case of quantitative results the extrapolation was corrected for differences in molecular weight.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors, ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-012-00-1	bentazone (ISO); 3-isopropyl-2,1,3-benzothiadiazine-4-one-2,2-dioxide	246-585-8	25057-89-0	Acute Tox. 4* Eye Irrit. 2 Skin Sens. 1 Aquatic Chronic 3	H302 H319 H317 H412	GHS07 Wng	H302 H319 H317 H412			
Dossier submitters proposal	613-012-00-1	bentazone (ISO);3-isopropyl-2,1,3-benzothiadiazine-4-one-2,2-dioxide	246-585-8	25057-89-0	**Modify:** Acute Tox. 4 **Retain:** Skin Sens. 1 **Add:** Repr. 2 **Remove:** Aquatic Chronic 3	**Modify:** H302 **Retain:** H317 **Add:** H361d **Remove:** H412	**Retain:** GHS07 Wng **Add:** GHS08	**Modify:** H302 **Retain:** H317 **Add:** H361d **Remove:** H412		Oral: ATE = 1640 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and COM	613-012-00-1	bentazone (ISO);3-isopropyl-2,1,3-benzothiadiazine-4-one-2,2-dioxide	246-585-8	25057-89-0	Repr. 2 Acute Tox. 4 Eye Irrit. 2 Skin Sens. 1	H361d H302 H319 H317	GHS07 GHS08 Wng	H361d H302 H319 H317		Oral: ATE = 1640 mg/kg bw	

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route		Yes
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation		Yes
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity		Yes
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Data conclusive but not sufficient for classification	Yes
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

In the meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances of November 1997 is agreed to classify the substance as Xn; R22 : Xi; R36 : R43 : R52-53 (ECBI/52/97 - Rev.1).

Bentazone is currently included in Annex VI of Regulation (EC) No 1272/2008 with Index Number 613-012-00-1 (CAS Number 25057-89-0) and classified as Acute Tox. 4* (H302), Eye Irrit. 2 (H319), Skin Sens. 1 (H317) and Aquatic Chronic 3 (H412).

A peer review of the pesticide risk assessment of the active substance bentazone as required by Commission Regulation (EU) No 1141/2010 as amended by Commission Implementing Regulation (EU) No 380/2013 is available (EFSA, 2015). Bentazone is proposed by the EFSA peer review to be classified as toxic for reproduction category 2 in accordance with the provisions of Regulation (EC) No 1272/2008.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level.

Reason for a need for action at Community level:

Change in existing entry due to new interpretation/evaluation of existing data.

Regarding environmental classification bentazone is assessed as not rapidly biodegradable and there are chronic data for algae/aquatic plants, aquatic invertebrates and fish. The lowest NOEC is above 1 mg/L. According to Table 4.1.0(b)(i) there is no need to classify for chronic aquatic toxicity, and the current classification as Aquatic Chronic 3 is to be removed.

5 IDENTIFIED USES

Bentazone acts as a selective post-emergent herbicide against broadleaved weeds in a broad range of crops, including cereals, maize, legume vegetables (pulses), bulb vegetables and forage crops (alfalfa, clover).

6 DATA SOURCES

The Renewal Assessment Report (RAR) on the renewal of the approval of the active substance bentazone of the EU (January 2015) and the Peer Review Report on Bentazone of EFSA (EFSA Journal 2015;13(4):4077) was used. Data on bentazone were collected from publically available data through a search using several databases including PubMed, EPA's ECOTOX knowledgebase and ToxNet. As there were two applicants multiple datasets are available for some parameters.

There is no REACH registration dossier of this substance (17 January 2019).

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	PAI: solid, white crystals (99.8 %) TGAI: yellow powder (97.6 %) Technical concentrate: red-brown clear liquid	Kroehl, 2011e Hogg, 2001a Kroehl, 2011f	visual assessments

Property	Value	Reference	Comment (e.g. measured or estimated)
	(644.9 g/L)		
Melting/freezing point	139° C (99.8 %, onset temperature) 139-141° C (99.8 %) (132.5 –138.1) ± 0.5°C	Kroehl, 2011d Jackson, 2001	measured measured
Boiling point	decomposition prior to boiling (99.8 %)	Tuerk, 1994	measured
Relative density	1.405 at 20°C 1.368 at 20 ± 0.3 °C	Kroehl, 1994a Hogg, 2001a	measured
Vapour pressure	4.9 x 10^{-4} Pa at 20°C (99.6 %) 1.7 x 10^{-4} Pa at 20°C (100 %)	Kaestel, 1999c Guckel, 1988	measured estimated
Surface tension	0.5 %: 69.2 mN/m (99.8 %) 2.0 %: 70.0 mN/m (99.8 %) 0.421 g/L: 68.9 mN/m (> 95 %)	Kroehl, 1994a Mullee and Bartlett, 1994	measured measured
Water solubility	(99.8%) pH 4: 3.0 g/L at 20° C pH 7: 7.7 g/L at 20° C pH 9: 17 g/L at 20° C (99.9%) pH 4: 1837 mg/L at 20° C pH 7: 7112 mg/L at 20° C pH 9: 5627 mg/L at 20° C	Class, 2001a Hogg, 2001b	measured measured
Partition coefficient n-octanol/water	(99.6%) deionized water: log P_{OW}: 1.49 at 20° C buffer pH 4: log P_{OW}: 1.54 at 20° C buffer pH 7: log P_{OW}: -0.94 at 20° C buffer pH 9: log P_{OW}: -1.32 at 20° C (99.6%) buffer pH 5: log P_{OW}: 0.77 at 22° C buffer pH 7: log P_{OW}: -0.46 at 22° C buffer pH 9: log P_{OW}: -0.55 at 22° C	Daum, 2000e Daum, 2000b Keller, 1986	measured measured
Volatility, Henry's law constant	2.108 x10^{-6} Pa/m^3/mol at 20°C 1.078 *10^{-4} Pa/m3/mol 7.2 x 10^{-5} Pa/m^3/mol	Brem, 2000a Hogg, 2001b	calculation calculation
Flash point	not applicable for solid		
Flammability	not flammable not classified as highly flammable	Gundrum, 2012a Jackson, 2001	measured
Explosive properties	not considered to be explosive not explosive when exposed to mechanical stress (shock and friction) not explosive when exposed to thermal stress.	Gundrum, 2012a Krips, 1995 Loffler, 1994 Tremain and Bartlett, 1994	measured
Self-ignition temperature	Auto-ignition temperature: 550 °C	Loeffler, 1994b	measured
Oxidising properties	not oxidising	Gundrum, 2012a Hogg, 2001a	measured measured

Property	Value	Reference	Comment (e.g. measured or estimated)
Granulometry			
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	pKa: 3.51 pKa: 2.50	Daum, 2000c Hogg, 2001b	measured measured
Viscosity	9.0 mPa × s. The viscosity remains unchanged after 2 years shef-life	Kaestel, 2005a Keller, 2011a	measured

* As summarized in the Final addendum to the RAR for Bentazone (as published by EFSA in January 2015) and the conclusion on the peer review of the pesticide risk assessment of the active substance bentazone (as published by EFSA on 20 April 2015; https://www.efsa.europa.eu/en/efsajournal/pub/4077)

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

As summarised and concluded in the Final addendum to the Renewal Assessment Report for Bentazone, January 2015. For description of the studies mentioned see Annex I.

Absorption

The toxicokinetic studies have shown that after oral administration bentazone is absorbed rapidly and to a large extent (> 80% of administered radioactivity). Bentazone levels were highest 0.5 hours after dosing. At this time, highest concentrations were confined to the gastro-intestinal tract, kidney, thyroid and plasma. 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. Bentazone has a low bioavailability after dermal administration (< 2%).

Distribution

Assessment of tissue residues and analysis of their time course did not reveal evidence for compound accumulation. Mean tissue residue values were all at or below the limit of measurement.

Metabolism

Bentazone is poorly metabolized with the parent compound being the predominating excretion product (81.4% and 85.3% of the administered dose in males and females, 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.

Excretion

Excretion of radiolabelled bentazone was rapid and occurred mainly via the urine (approximately 90% of the administered radioactivity). Most of renal elimination was complete within the first 24 hours after dosing

(>80% of the total eliminated dose). Only traces (1% to 2%) were excreted in the faeces, bile or exhaled in the air. Repeated administration did not significantly alter the pattern of elimination. There was an indication of a non-linear region of plasma levels over time. The plasma kinetic study and a mechanistic study to elucidate the elimination path showed that bentazone is actively secreted via the saturable renal Organic Anion Transporter, and that saturation of excretion starts at dose levels between 84.7 and 165.9 mg/kg bw (calculated as bentazone sodium salt).

10 EVALUATION OF HEALTH HAZARDS

The health hazards of bentazone were assessed in the Renewal Assessment Report (RAR), (January, 2015) concerning the renewal of the approval of the active substance bentazone under Regulation (EC) 1107/2009. The RAR as well as the Conclusion on the peer review of the pesticide risk assessment of the active substance bentazone (EFSA Journal 2015;13(4):4077) are publically available.

The summaries included in this proposal are copied from the RAR (and its addenda and assessment reports when these contain updated information). The RAR includes several studies from the original DAR and therefore references to the DAR are also included in this report. For an overview of the hazard property being evaluated, all reliable information relating to that property has been summarised in a table. Detailed information is included for the key studies used to derive the classification. For more details on the studies see Annex I, where the description of the studies from the RAR and its addenda, i.e. RAR Volume 3 B 6 – Annex B: B.6 Toxicology and metabolism are included.

Acute toxicity

10.1 Acute toxicity - oral route

There were no new animal studies on acute oral toxicity study of bentazone since the RAR dated January 2015. However, as there is currently a minimal classification for acute oral toxicity, the available studies were assessed to determine the correct classification. For description of the studies mentioned in the Table 9 see Annex I.

Table 9: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
The test procedure to a great extent followed OECD 401 and there were no deviations to Directive 92/69/EEC, part B, December 29, 1992. GLP: No.	Male and female Wistar rats; 5M and 5 F/group	Bentazone (purity 93.9%, batch no. not available)	Administered by gavage as 0.5% aqueous CMC solution at dose levels of 825; 1,210; 1,780 and 2,610 mg/kg bw	1640 mg/kg bw for males and females.	Anonymous 1983 (Doc. No. 83/114)[a] Klimisch score: 2
The test procedure to a great extent complied with OECD guideline 401 and there were no deviations from	Male and female Wistar rats; 5M and 5F/group.	Bentazone technical (batch no. not available)	Administered as 0.5% aqueous CMC solution by oral gavage at dose levels 562; 825; 1,210; 1,780 and 2,610 mg/kg bw	1710 (1340 - 2460 95% C.I.) mg/kg bw for male and female animals	Anonymous 1983 (Doc. No. 83/113) [a] Klimisch score: 2

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Directive 92/69/EEC, part B, December 29, 1992. GLP: No					
The study was performed prior to implementation of specific test 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	Male and female Sprague-Dawley rats (5M and 5F/group)	Bentazone technical (batch no. not available)	Administered as 0.8% aqueous CMC solution by oral gavage at dose levels of 500; 640; 800; 1000; 1,250; 1,600 and 2,000 mg/kg bw	1220 (1056 - 1409 95% C.I.)	Anonymous 1973 (Doc. No. 73/022) [a] Klimisch score: 4
The study was performed prior to implementation of specific test guidelines. GLP: No	Male and female Wistar rats; 5M and 5F/group.	Bentazone sodium salt	Administered as 0.8% aqueous CMC solution by oral gavage at dose levels of 800; 1000; 1250; 1600 and 2000 mg/kg bw	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.	Anonymous 1973 (Doc. No. 73/023) [a] Klimisch score: 4
This study was performed prior to implementation of specific test 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	Sprague-Dawley (CRJ:SD) rats; 10M and 10F/group.	Bentazone (purity: 94.6%, batch no. 270 778)	administered as 0.5% aqueous CMC solution by gavage at dose levels of 1500; 1800; 2160; 2592; 3110 and 3732 mg/kg bw	2340 (2208 - 2480 C.I.) mg/kg bw for male and 2470 (2058 - 2964 C.I.) mg/kg bw for female animals.	Anonymous (1978) (Doc. No. 78/053) [a] Klimisch score: 4

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
report. The LD_{50} values were calculated on day 7 mortality rate (However, no animal died between day 7 and scheduled termination). GLP: No					
Study was performed prior to implementation of specific test guidelines. GLP: No	Male and female Sprague-Dawley rats; 5M and 5F/group)	Bentazone (former name thianon; batch no. not available; purity not determined)	Administered as 2 - 16% aqueous tragacanth suspension by oral gavage at dose levels of 200; 400; 800; 1000; 1250 and 1600 mg/kg bw	850 mg/kg bw for male and female animals.	Anonymous 1969 (Doc. No. 69/0013) [a] Klimisch score: 4
Study was performed prior to implementation of specific test guidelines. No: GLP	Male and female Sprague-Dawley rats (source: Ivanovas, KiSlegg, Germany); 5M and 5F/group	Bentazone technical (batch no. not available; purity not determined)	Administered as 8% aqueous CMC suspension by oral gavage at dose levels of 400; 500; 640; 800; 1000; 1,250; 1600 and 2000 mg/kg bw.	1050 (847 - 1302 confidence interval) mg/kg bw for male and female animals	Anonymous 1972 (Doc. No. 72/051) [a] Klimisch score: 4
The study was performed prior to implementation of specific test guidelines. GLP: no Limited study design (n=2)	Male and female rabbit (strain not specified); 1M and 1F/group.	Bentazone technical (batch no. not available)	Administered as 2.5; 5 or 20% aqueous tragacanth suspension by oral gavage at dose levels of 100; 250; 500; 1000 and 2000 mg/kg bw.	About 750 mg/kg bw.	Anonymous 1969 (Doc. No. 69/005) [a] Klimisch score: 4
Published literature.	Male and female NZW-rabbit;	Bentazone		LD_{50} of 1139 mg/kg bw was calculated with respiratory, cardiac and central nervous symptoms occurring	Neuschl J and Kacmar P, 1993 (Doc. #93/11411)[a] Klimisch score: 4
No specific test guidelines did exist at that time. No GLP	Male and female Guinea pigs (strain not specified); 5M and 5F/group	Bentazone technical ("free acid", batch no. not available)	Orally administered as 4 - 16% aqueous CMC solution al dose levels at 400; 800; 1200; 1600 or 3200 mg/kg bw	1100 mg/kg bw for male and female animals	Anonymous 1991 (Doc. No. 91/10147) [a] Klimisch score: 4
No specific test	Male and female	Bentazone	Orally administered	LD_{50} was found	Anonymous 1974

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
guidelines did exist at that time. No GLP.	Guinea pigs; 5M and 5F/group.	sodium salt (batch no. not available)	as 6.4 - 16% aqueous CMC solution at dose levels of 640; 800; 1000; 1250 and 1600 mg/kg bw.	to be about 1100 mg bentazone sodium salt/kg bw equivalent to about 1000 mg bentazone acid/kg bw for male and female animals	(Doc. No. 74/035) [a] Klimisch score: 4
No specific test guidelines did exist at that time. No GLP.	8 male and 3 female Beagle dogs; 2 animals/group.	Bentazone (batch no. not available; purity not determined)	Administered as aqueous tragacanth solution (1 - 20%) by oral gavage at dose levels of 50; 100; 250; 500; 1000 and 2000 mg/kg bw.	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.	Anonymous 1970 (Doc. No. 70/017) [a] Klimisch score: 4
No specific test guidelines did exist at that time. No GLP	Two to six cats tested per sex and dose.	Bentazone technical (batch no. not available)	Administered as aqueous tragacanth solution (5 - 20%) by oral gavage at dose levels of 250; 500; 1000 and 2000 mg/kg bw	LD_{50} was found to be about 500 mg/kg bw for male and female cats.	Anonymous 1970 (Doc. No. 70/016) [a] Klimisch score: 4

[a] As summarised in the Final addendum to the Renewal Assessment Report for Bentazone, January 2015.

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Numerous studies on the acute oral toxicity of bentazone have been carried out in various species. A summary of the results of these studies is given in Table 9: Summary table of animal studies on acute oral toxicity. In most of these experiments, bentazone was administered in its free acid form. Testing of the sodium salt is indicated in the table above.

In summary, it can be stated that bentazone has a low or moderate acute oral toxicity in various animal species. The use of the sodium salt of bentazone did not result in a higher toxicity. The calculated oral LD_{50} value in rats varied from 850-2470 mg/kg bw. The lowest calculated LD_{50} value in guinea pig is 1000 mg/kg bw, in rabbit about 750-1139 mg/kg bw, in cats 500 mg/kg bw and in dogs > 500 mg/kg bw.

10.1.2 Comparison with the CLP criteria

Substances can be allocated to one of four toxicity categories based on acute toxicity by the oral route according to the numeric criteria shown in Annex I, Table 3.1.1 in Regulation (EC) 1272/2008 on CLP. Following this table, lowest calculated oral LD_{50} value (derived from an acceptable study) is 1640 mg/kg bw in rats (Anonymous, 1983 (Doc No. 83/114)), resulting in classification as Acute Tox. 4 (criterion LD_{50} 300 – 2000 mg/kg bw). Also the lowest LD_{50} values in other animal species (though most studies being considered as supplemental data only) fall in Acute Tox. 4.

An LD_{50} value of 1640 mg/kg bw is suggested as ATE for acute oral toxicity.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Bentazone should be classified as Acute Tox. 4 (H302: Harmful if swallowed).

It is proposed to assign an ATE of 1640 mg/kg bw for acute oral toxicity.

10.2 Acute toxicity - dermal route

Not evaluated in this dossier.

10.3 Acute toxicity - inhalation route

Not evaluated in this dossier

10.4 Skin corrosion/irritation

Not evaluated in this dossier

10.5 Serious eye damage/eye irritation

Not evaluated in this dossier

10.6 Respiratory sensitisation

Not evaluated in this dossier.

10.7 Skin sensitisation

No new animal studies on skin sensitisation were submitted for the renewal of bentazone since the original DAR dated September 1996. However, the criteria for skin sensitisation now differentiate between category 1, 1A and 1B. For description of the studies mentioned in the Table 10, see Annex I.

Table 10: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
The study was performed according to OECD 406 (Maximization test) GLP: yes	20 female Pirbright White Guinea pigs	bentazone (purity: 94.0%; batch no. MS 2 F 22)	intradermally applied as aqueous solution [5% in aqua dest. or in Freund's adjuvant/aqua dest. (1:1)]	Sensitising to skin: 12/20, 6/20 and 16/20 animals tested positive at 1st, 2nd and 3rd challenge	Anonymous 1986 (Doc. No. 86/195)[a] Klimisch score: 1
The study was performed according to OECD 406 (OET). GLP: yes.	eight female Pirbright White guinea pigs	Bentazone sodium salt formulation (600 g/L; batch no. WH 4976)		Sensitizing at 50% aqueous dilution: 2/8 and 3/8 animals tested positive at 1st and 2nd challenge Not sensitizing at 2% and 10% aqueous dilution	Anonymous 1986 (Doc. No. 86/221)[a]

[a] As summarised in the Final addendum to the Renewal Assessment Report for Bentazone, January 2015.

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

The maximization test used test groups consisting of 20 animals for the treated group and 10 animals for the controls (3 control groups each for one challenge) (Anonymous 1986, Doc. No. 86/195). Three challenges instead of only one were included. After intradermal induction (5%), 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. (concentration not stated) caused incrustation (which resulted from the intradermal induction) in addition to distinct erythema and edema.
After the first challenge (50% in aqua dest.), 10/20 test animals exhibited distinct erythema. Eight of these ten animals had also slight edema. 2/2 0 test animals only showed slight erythema.
In the control group 1, no skin reactions were noted.

After the second challenge (50% in aqua dest.), 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 (50% in aqua dest.), distinct erythema and slight edema were observed in 9/20 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 11.

Table 11: 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

In a second study with sodium bentazone in Guinea pigs using the open epicutaneous test (OET), in the induction phase (20 applications (0.1 ml per 8 cm^2) of 600g/L sodium bentazone, and 2%, 10% and 50% dilutions in aqua dest.) no skin reactions were noted in animals of the control and test groups (Anonymous 1986, Doc. No. 86/221). The number of animals with skin findings after the first challenge (day 28) and after the second challenge (day 42) is collated in Table 12 (0.025 ml/ 2 cm^2).

Table 12: 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.

10.7.2 Comparison with the CLP criteria

Positive skin reaction in 12/20 animals after first challenge, 6/20 after second challenge and 16/20 after third challenge were observed in a maximization study after intradermal induction with 5% bentazone. Classification is required when a positive response is observed in 30% or more animals in a maximisation test at >1% intradermal induction dose. Therefore, these results require classification. Classification in category 1A is required when a positive reaction is observed in 60% or more of the animals after intradermal induction with 0.1% - 1%. However, the tested concentration was above 1%. However, seen the high level of positive responses at 5% (up to 80%), a response at or above 60% at 1% cannot be fully excluded. Therefore, no sub-category can be assigned and classification in category 1 is justified based on this study.

No criteria are available for classification based on an open epicutaneous test and especially not for sub-categorisation. For application concentrations of 50% (of 600 g/L = 30%) 2/8 (25%) after first challenge and 3/8 (38%) after second challenge showed a positive response. However, the absence of a dose effect relation puts some doubt on the results. Comparison with the criteria for a Buehler test would indicate a need for classification in category 1 (above 15% positive responders) and more specifically in category 1B as a no response was observed after induction with 6%. However, the applied OET is not a Buehler test, therefore some uncertainties remain regarding the classification for skin sensitisation.

Overall, the available data indicate a requirement for classification. However, these data are not conclusive for sub-categorisation and in that case skin sensitisers shall be classified in Category 1.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Bentazone should be classified as Skin Sens. 1 (H317 May cause an allergic skin reaction).

10.8 Germ cell mutagenicity

Not evaluated in this dossier.

10.9 Carcinogenicity

Not evaluated in this dossier. However, a summary is provided to support the evaluation of the endpoint reproductive toxicity. Carcinogenicity studies (Anonymous 1985 (Doc. No. 85/433); Anonymous 1985 (Doc.

No. 85/432) show that a prolonged blood coagulation time and a decreased hematocrit and hemoglobin content were the most frequent findings observed. In other carcinogenicity studies (Anonymous 1974 (Doc. No. 74/004); Anonymous 1974 (Doc. No. 74/041); Anonymous 1978 (Doc. No. 78/034) hematological and clinical chemistry parameters were not investigated.

Table 13: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
No specific guideline GLP: No Rat, Sprague-Dawley, 50/sex/dose	Bentazone technical (batch no. and purity not submitted) Oral (via diet) 0; 100; 350 and 1600 ppm, equivalent to 0, 5, 17 and 76 mg/kg bw/day (means for males and females). 2-year	1600 ppm: reduced bw and food consumption; increased organ weights (males and females). NOAEL 350 ppm LOAEL 1600 ppm	Anonymous 1974 (Doc. No. 74/004)[a] Klimisch score: 2
OECD 453 GLP: yes Rat, Fischer 344 Dus/Crj (SPF), 50/sex/dose	Bentazone (purity 93.9%; batch no. N 169) Oral (via diet) 0; 200; 800 and 4000 ppm, equivalent to intake for 104 weeks: 0, 9/11, 35/45, 180/244 (m/f) mg/kg bw/day; intake for 26 weeks: 0, 9/12, 39/48, 197/249 (m/f) mg/kg bw/day; intake for 52 weeks: 0, 12/14, 47/55, 233/274 (m/f) mg/kg bw/day. 2-year	4000 ppm: reduced bw (males and females); 800 ppm and 4000 ppm: effect on blood coagulation, impairment of liver and kidney function indicated by changes in clinical chemistry and organ weights. NOAEL 200 ppm LOAEL 800 ppm	Anonymous 1985 (Doc. No. 85/433)[a] Klimisch score: 1
OECD 453 GLP: yes Mouse, CRJ:B6C3Fl,50/sex/dose	Bentazone (purity: 93.9%; batch no. N 169). Oral (via diet) 0; 100; 400 and 2000 ppm; equivalent to 0, 12/12, 47/48, 242/275 (m/f) mg/kg bw/day. 2 years	2000 ppm: transient reduction of bw gain (males) > 400 ppm: impaired blood coagulation; increased testicular calcification (Questionable effect, not confirmed in other studies); proliferative lesions in the liver (females). NOAEL 100 ppm LOAEL 400 ppm	Anonymous 1985 (Doc. No. 85/432)[a] Klimisch score: 1
No specific guideline GLP: No Mouse, Swiss Webster, 50/sex/dose	Bentazone technical (batch no. not available) Oral (via diet) 0; 100; 350 and 1600 ppm, equivalent to 0, 15; 52 and 237 mg/kg bw/day (means for males and females). 18 months	High mortality (> 50%) in test and control groups. 1600 ppm: reduced food consumption and bw, organ weight changes. NOAEL 350 ppm LOAEL 1600 ppm	Anonymous 1974 (Doc. No. 74/041)[a] Klimisch score 4

No specific guideline GLP: No Mouse, CFLP, 40 sex/dose.	Bentazone technical (batch p. 195.75) Oral (via diet) 0; 100; 350 and 1600 ppm, equivalent to 0, 8.4/9.5, 29.7/34.3, 138.4/152.9 (m/f) mg/kg bw/day. 88-92 weeks	No substance-induced findings. NOAEL 1600 ppm LOAEL –	Anonymous 1978 (Doc. No. 78/034)[a] Klimisch score 4

[a] As summarised in the Final addendum to the Renewal Assessment Report for Bentazone, January 2015.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Long-term studies in rats and mice did not reveal a carcinogenic potential of bentazone although the compound may have caused proliferative lesions in the liver of female mice. In long-term studies in both species, an impairment of blood coagulation and minor effects on liver and kidney were observed. Frequently, a reduced body weight or body weight gain and a diminished food consumption were noted. The lowest NOAEL for chronic toxicity of about 9 mg/kg bw/day was derived from a study in Fischer 344 rats. In a previous rat study, a similar NOAEL of approximately 17 mg/kg bw/day had been established. In the most recent chronic feeding study in mice, the NOAEL was about 12 mg/kg bw/day.

10.9.2 Comparison with the CLP criteria

Not evaluated in this dossier. However, a summary is provided to support the evaluation of the endpoint reproductive toxicity.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Not evaluated in this dossier.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

No new reproductive toxicity studies were submitted for the renewal of bentazone since the original DAR dated September 1996. Table 14 summarises the animal studies on adverse effects on sexual function and fertility. For description of the studies mentioned in the Table 14, see Annex I.

Supplementary studies from published literature on spermatogenesis and hormonal activity are included in Table 15 which were not included in the previous DAR dated September 1996.

Table 14: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
3-generation toxicity study. Rat, Sprague-Dawley,	Bentazone technical (purity not available, batch no. not available). Oral (via diet)	Parental toxicity: No effects Sexual function/fertility: no effects Developmental toxicity: no effects	Anonymous 1973 (Doc. No. 73/010) [a] Klimisch

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
20/sex/dose No specific test guidelines. No GLP.	0, 20, 60 and 180 ppm, equivalent to P: 0, 1.6/2.5, 4.7/7.3, 15.0/21.9 (m/f) mg/kg bw/day F1: 1.6/2.4, 4.5/7.2, 14.1/21.5 (m/f) mg/kg bw/day F2: 1.6/2.1, 4.8/6.6, 14.2/20.5 (m/f) mg/kg bw/day F3: 2.2/2.0, 6.4/6.4, 19.4/19.8 (m/f) mg/kg bw/day	Parental: NOAEL 180 ppm (highest dose tested) Developmental: NOAEL 180 ppm Fertility: NOAEL 180 ppm	score: 3
Two-generation study Rat, Wistar/HAN, 2-5/sex/dose OECD 416 GLP: yes	Bentazone (purity: 97.8%, batch no. N 187) Oral (via diet) 0; 200; 800 and 3200 ppm, equivalent to P (pre-pairing period, day to day 70): 0, 14.8/17.0, 58.5/66.9, 238/268.9 (m/f) mg/kg bw/day P (after pairing, day 1 to day 21/22): 0, 10.3/14.7, 40.7/60.7, 164.3/246.7 (m/f) mg/kg bw/day P (lactation period, day 1 to day 14): 0, 29.7, 111.0, 472.7 mg/kg bw/day for females F1 (pre-pairing period, day 1 to day 123): 13.7/15.9, 56.9/64.4, 227/261.6 (m/f) mg/kg bw/day F1 (after pairing period, day 1 to day 25, males/gestation period, day 0 to day 21, females): 10.0/14.3, 40.8/59.3, 168.3/238.7 (m/f) mg/kg bw/day F1 (lactating period, day 1 to day 14): 29.0, 121.3, 492.0 mg/kg bw/day for females	Parental toxicity: 3200 ppm: decreased bw F1 males (during prepairing and postpairing periods) and F0/F1 females (all periods); reduced feed consumption between PND 1-4 (females: -17.2 (63%))* 800 ppm: reduced feed consumption between PND 1-4 (F0 females: -14.2 (52%)**, body weight gain between PND 1-4 (F0 females: -4.8 (29%) ** 200 ppm: no effects Fertility/sexual function: no adverse effects Developmental toxicity: F1: 3200 ppm: No body weight effect on PND1. Reduced body weight (day 7: -8.8%). 800 ppm: No body weight effect on PND1, reduced body weight (PND 4: -11.9%, PND 7: -10.2%), reduced food consumption between PND 1-4 (38.4%). Body weight are outside the range of historical data. 200 ppm: no effect F2: 3200 ppm: No body weight effect on PND1. Reduced body weight (PND 4: -5.5, PND 7: -4.1, PND 14: --8.1, PND 21: -8.3). Body weight are within the range of historical data. 800 ppm: mean pup weight (PND 4: -3.3, PND 14: -2.9, PND 21: -3.9). Body weight are within the range of historical data. 200 ppm: no effect.	Anonymous 1989 (Doc. No. 89/0068)[a] Klimisch score: 1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Parental: NOAEL 200 ppm (~22 mg/kg bw/day), critical effect reduced bw and feed consumption. Offspring: NOAEL 200 ppm (~22 mg/kg bw/day), critical effect reduced body weight of the F1 and F2 pups. Fertility: NOAEL 3200 ppm (163.4 mg/kg bw/day); reproductive functions not impaired.	

[a] As summarised in the Final addendum to the Renewal Assessment Report for Bentazone, January 2015.

* Taking only those dams of the 3200 ppm group into account which had a total litter loss or whose pups showed reduced pup weights over the period of PND 4-7 which are #182, 185, 189, 191 and 194

** Taking only those dams of the 800 ppm group 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

Table 15: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Spermatogenesis in mice	Bentazone (purity not reported)	Oral exposure via drinking water for 100 days to the very low dose of 21 µg/kg bw/day	The results show that there were no effects of bentazone on spermatogenesis at the tested concentration.	Garagna et al. 2005 [a]
Two in vitro assays: Recombinant yeast screen (YES-assay and YAS-assay); Xenopus oocytes	Bentazone		Bentazone induced anti-androgenic transcriptional activity at 500 – 1000 µM in the recombinant yeast screen used to detect receptor mediated (anti-) androgenic activity (YAS-assay) after co-incubation with 2.5 nM dihydrotestosteron. An androgenic transcriptional activity was not observed. Bentazone showed no effect in the recombinant yeast screen used to detect receptor mediated (anti-)estrogenic activity (YES-assay). In addition, no effect of bentazone was observed in the ovulation assay.	Orton et al. 2009 [a]
QSAR models to address ED effects mediated			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	Roncaglioni et al. 2008 [a]

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
through the estrogen receptor (ER); Japanese METI database			activity.	
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.	Bentazone		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.	Kojima 2004 [a]
Recombinant human androgen receptor AR (rhAR)	Bentazone (purity 99.9%, dissolved in ethanol)		Bentazone showed no 3H-DHT displacement even at the highest soluble concentration, indicating no binding affinity to the hAR.	Bauer 2002 [a]
E-Screen Assay based on the human breast cancer cell line MCF-7	Bentazone		Bentazone showed no estrogen-receptor mediated activity in the *in vitro* test system.	Bitsch 2002 [a]

[a] As summarised in the Final addendum to the Renewal Assessment Report for Bentazone, January 2015.

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Two multigeneration studies in rats did not reveal adverse effects on the reproductive functions. Bentazone showed no effect on spermatogenesis. It is however noted that the dose levels in the 3-generation study were quite low. The effect of bentazone as androgen antagonist in the YAS-assay was only observed at non-physiological high concentrations. According to Bauer et al. 2002, a direct binding of bentazone to the hAR does not take place up to high concentrations. Anti-androgenic activity was not confirmed in any higher tier study neither in mice nor rats. Nor were adverse effects on spermatogenesis observed in the study in mice at

low dose levels and reproductive performance was not affected in rats. The available repeated dose toxicity studies, as summarised in Annex I, do not show substance related effects on the reproductive organs at the tested dose levels. It is therefore concluded that bentazone shows no effect on sexual function and fertility.

10.10.3 Comparison with the CLP criteria

No classification is warranted for adverse effect on sexual function and fertility as no effect on fertility or on the reproductive organs was observed in any of the available studies.

10.10.4 Adverse effects on development

No new studies were submitted. In the European Commision Final Review Report of bentazone the effect of pup weight in the 2-generation toxicity study in rats (Anonymous 1989, Doc. No. 89/0068) 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, a re-evaluation of the study has been performed, 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 (RAR 2015).

Further, it is stated in the conclusion document of the pesticide peer review of EFSA (2015) that: "Developmental effects, such as increased post-implantation loss, reduced number of live foetuses and retarded foetal development were observed in a rat developmental study in the absence of clear maternal toxicity, supported by evidence in other developmental studies in rats. Based on these developmental effects the experts of EFSA suggested that classification regarding reproductive toxicity, Repr. Cat. 2; H361d 'suspected of damaging the unborn child' may be appropriate (no classification regarding this endpoint is included in the current harmonised classification according to Regulation (EC) No 1272/2008). The developmental NOAEL is 100 mg/kg bw per day and the maternal NOAEL is 250 mg/kg bw per day in rats."

Table 16: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
3-generation toxicity study. Rat, Sprague-Dawley, 20/sex/dose No specific test guidelines. No GLP.	Bentazone technical (purity not available, batch no. not available). Oral (via diet) 0, 20, 60 and 180 ppm, equivalent to P: 0, 1.6/2.5, 4.7/7.3, 15.0/21.9 (m/f) mg/kg bw/day F1: 1.6/2.4, 4.5/7.2, 14.1/21.5 (m/f) mg/kg bw/day F2: 1.6/2.1, 4.8/6.6, 14.2/20.5 (m/f) mg/kg	Parental toxicity: No effects Sexual function/fertility: no effects Developmental toxicity: no effects Parental: NOAEL 180 ppm (highest dose tested) Developmental: NOAEL 180 ppm Fertility: NOAEL 180 ppm No adverse effects on adults nor offspring.	Anonymous 1973 (Doc. No. 1973/010) [a] Klimisch score: 3

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	bw/day F3: 2.2/2.0, 6.4/6.4, 19.4/19.8 (m/f) mg/kg bw/day		
Two-generation study Rat, Wistar/HAN, 2-5/sex/dose OECD 416 GLP: yes	Bentazone (purity: 97.8%, batch no. N 187) Oral (via diet) 0; 200; 800 and 3200 ppm, equivalent to P (pre-pairing period, day to day 70): 0, 14.8/17.0, 58.5/66.9, 238/268.9 (m/f) mg/kg bw/day P (after pairing, day 1 to day 21/22): 0, 10.3/14.7, 40.7/60.7, 164.3/246.7 (m/f) mg/kg bw/day P (lactation period, day 1 to day 14): 0, 29.7, 111.0, 472.7 mg/kg bw/day for females F1 (pre-pairing period, day 1 to day 123): 13.7/15.9, 56.9/64.4, 227/261.6 (m/f) mg/kg bw/day F1 (after pairing period, day 1 to day 25, males/gestation period, day 0 to day 21, females): 10.0/14.3, 40.8/59.3, 168.3/238.7 (m/f) mg/kg bw/day F1 (lactating period, day 1 to day 14): 29.0, 121.3, 492.0 mg/kg bw/day for females	<u>Parental toxicity</u>: 3200 ppm: decreased bw F1 males (during prepairing and postpairing periods) and F0/F1 females (all periods); reduced feed consumption between PND 1-4 (females: -17.2 (63%))* 800 ppm: reduced feed consumption between PND 1-4 (F0 females: -14.2 (52%)**, body weight gain between PND 1-4 (F0 females: -4.8 (29%) ** 200 ppm: no effects <u>Fertility/sexual function</u>: no adverse effects <u>Developmental toxicity</u>: F1: 3200 ppm: No body weight effect on PND1. Reduced body weight (day 7: -8.8%). 800 ppm: No body weight effect on PND1, reduced body weight (PND 4: -11.9%, PND 7: -10.2%), reduced food consumption between PND 1-4 (38.4%). Body weight are outside the range of historical data. 200 ppm: no effect F2: 3200 ppm: No body weight effect on PND1. Reduced body weight (PND 4: -5.5, PND 7: -4.1, PND 14: --8.1, PND 21: -8.3). Body weight are within the range of historical data. 800 ppm: mean pup weight (PND 4: -3.3, PND 14: -2.9, PND 21: -3.9). Body weight are within the range of historical data. 200 ppm: no effect. Parental: NOAEL 200 ppm (~22 mg/kg bw/day), critical effect reduced bw gain and food consumption Offspring: NOAEL 200 ppm (~22 mg/kg bw/day), critical effect reduced body weight of the F1 and F2 pups Fertility: NOAEL 3200 ppm (163.4 mg/kg bw/day); reproductive functions not impaired	Anonymous 1989 (Doc. No. 89/0068)[a] Klimisch score: 1
Teratogenicity	Bentazone sodium	<u>Maternal</u>:	Anonymous 1991

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
study (range-finding) Rat, Wistar, 5 females/dose	(batch no. B016) By oral gavage 0, 50, 150 and 450 mg/kg bw/day Gestation day 6-15	No adverse effects <u>Developmental and fetal findings</u>: 450 mg/kg bw/day: increased post-implantation losses 39.0% vs. 9.7% in control group), decreased fetal weight (6.4% on an individual basis and 8.5% on a litter basis). 150 mg/kg bw/day: increased post-implantation losses (29.3% vs. 9.7% in control group). 50 mg/kg bw/day: no effects	(Agrichem file no. R 463)[a]
Teratogenicity study. Rat, Wistar, 25 females/dose OECD 414 GLP: Yes	Bentazone sodium (batch no. B016) By oral gavage 0; 5; 30 and 180 mg/kg bw/day Gestation day 6-15 The study was extended by a supplementary dose group of 25 females receiving 360 mg/kg bw/day.	<u>Maternal:</u> 360 mg/kg bw/day: reduced food consumption (8.8%) during first half of the treatment period, reduced body weight from day 8 until day 21 p.c. (-4%). <u>Developmental and fetal findings:</u> 360 mg/kg bw/day: slight reduced body weight. The slight effects on food consumption and body weight in dams and pups are not considered adverse. Maternal: NOAEL 360 mg/kg bw/day Developmental: NOAEL 360 mg/kg bw/day.	Anonymous 1991 (Agrichem file no. R 22)[a] Klimisch score: 1
Teratogenicity study Rat, Wistar, 25 females/dose OECD 414 GLP: Yes	Bentazone (purity: 97.8%, batch no. N 187) By oral gavage 0 (vehicle control); 40; 100 and 250 mg/kg bw/day Gestation day 6-15	<u>Maternal:</u> 250 mg/kg bw/day: reduced food consumption between day 6 and 11. However as the effect was only slight (-5.6%) the effect was not considered to be adverse. <u>Developmental and fetal findings</u>: 250 mg/kg bw/day: increased post-implantation losses (14.4%), decreased fetal weight (-10.4%). Maternal: NOAEL 250 mg/kg bw/day Developmental: NOAEL 100 mg/kg bw/day.	Anonymous 1986 (Doc. No. 86/421)[a] Klimisch score: 1
Teratological study Rat, SD/CRJ, 23 females/dose No specific guideline GLP: Yes.	Bentazone (purity: 93.9%, batch no. N 169) By diet 0; 2000; 4000 or 8000 ppm, equivalent to 0, 162, 324, 631 mg/kg bw/day. Gestation day 0-21	<u>Maternal:</u> 631 mg/kg bw/day: alopecia (one animal), hematuria and brownish urine, depression, skin pallor, piloerection and nasal hemorrhage from day 19 of gestation onwards (four animals), reduced body weight (-6.5% at day 21), reduced food consumption (-37,5% at day 21), increased water consumption (18%), increased amniotic fluid weight (32%). 324 mg/kg bw/day: increased food (12.5% at day 21) and water consumption (11.6%), increased amniotic fluid weight (33%). 162 mg/kg bw/day: increased food consumption (11.5% at day 1).	Anonymous 1982 (Doc. No. 84/066)[a] Klimisch score: 3

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Developmental and fetal findings: 631 mg/kg bw/day: reduced body weight, petechiae in the liver, reduced ossification of cervical vertebrae. Maternal: NOAEL 162 mg/kg bw/day. Developmental: NOAEL 324 mg/kg bw/day.	
Teratogenicity study. Rabbit, Chinchilla rabbits, 16 females/dose OECD 414 GLP: yes	Bentazone (purity: 97.8%, batch no. N 187) By oral gavage 0; 75; 150 and 375 mg/kg bw/day Gestation day days 6 - 18	Maternal: 375 mg/kg bw/day: reduced food consumption (-6.2%), five aborted placentae were found on day 22 p.c., Developmental and fetal findings: 375 mg/kg bw/day: total post-implantation loss in one animal. NOAEL 150 mg/kg bw/day Developmental: NOAEL 150 mg/kg bw/day;	Anonymous 1987 (Doc. No. 87/058)[a] Klimisch score: 1
Study supplementary only			
Teratogenicity study. Rat, Sprague-Dawley, 20-32 females/dose Guidelines for reproduction studies for safety evaluation of drugs for human use (FDA, 1966) GLP: No	Bentazone technical (purity not available, batch no. not available) By oral gavage 0; 22.2; 66.7 and 200 mg/kg bw/day Gestation day 6-15	Study does not comply with current standards. Study supplementary only. Maternal: No effects. Developmental and fetal findings: 200 mg/kg bw/day: increased resorption rate (66.3% of all implantations), reduced body weight, increased number of runts (13.4%), increase in frequency of anasarca (11.5%). Teratogenic effects not reproducible (see Doc. No. 1978/039) for embryo-/fetotoxicity. Maternal: NOAEL 200 mg/kg bw/day Developmental: NOAEL 66.7 mg/kg bw/day	Anonymous 1971 (Doc. No. 71/0041) [a] Klimisch score: 4
Teratogenicity study. Rat, Sprague-Dawley, 26-29 females/dose. Guidelines for reproduction studies for safety evaluation of drugs for human use" (FDA, 1966	Bentazone (purity: 92.5%, batch no. not available) By oral gavage 0; 22.2; 66.7 and 200 mg/kg bw/day Gestation day 6-15	Study does not comply with current standards. Study supplementary only. No critical effects. Maternal: NOAEL 200 mg/kg bw/day Developmental: NOAEL 200 mg/kg bw/day.	Anonymous 1978 (Doc. No. 78/039) [a] Klimisch score: 3

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
GLP: No			
Teratogenicity study Rat Public literature.	Basagran (a bentazone-containing formulation; origin and purity not submitted), 0; 25; 90; 200 mg/kg bw/day, corresponding to 0; 12.0; 43.2 or 96 mg of bentazone/kg bw/day) By oral gavage. Gestation day 6, 8, 11, 14, 16	Study unacceptable due to inconsistency of the data reported. No information on maternal toxicity, only summary of gross-pathological examination, frequency of changes not reported. Maternal: no data given Developmental: Increased resorption rate, retardation of fetal development and incomplete ossification in all treated groups. No dose-response relationship. Inconsistency of data. No assessment possible.	El-Mahdi MM and Lofti MM 1988 [a] Klimisch score: 4
Teratogenicity study. Rabbit, Himalayan ChBB:HM, 15 females/dose Guidelines for reproduction studies for safety evaluation of drugs for human use" (FDA, 1966) GLP: No	Bentazone (purity 92.5%, batch no. not available) By oral gavage 0; 50; 100 and 150 mg/kg bw/day Single dose on gestation day 6, 8, 11, 14, 16, 18	Study does not comply with current standards. Study supplementary only. No critical effects. Maternal: NOAEL 150 mg/kg bw/day Developmental: NOAEL 150 mg/kg bw/day.	Anonymous 1984 (Doc. No. 84/048) [a] Klimisch score: 3

[a] As summarised in the Final addendum to the Renewal Assessment Report for Bentazone, January 2015.

* Taking only those dams of the 3200 ppm group into account which had a total litter loss or whose pups showed reduced pup weights over the period of PND 4-7 which are #182, 185, 189, 191 and 194

** Taking only those dams of the 800 ppm group 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

Table 17: Summary table of other studies relevant for developmental toxicity (i.e. studies with metabolite 8-OH-bentazone)

Type of study/data	Test substance,	Observations	Reference
OECD 414 GLP: yes	Metabolite: 8-OH-bentazone (purity: 99.9%; batch no. 108 746, lot no. L 47-213) By oral gavage 0; 40; 100 and 250 mg/kg	The study is considered supplementary only since the highest dose administered did not cause any signs of toxicity. No critical effects.	Anonymous 1993 (Doc. No. 93/10572)

Type of study/data	Test substance,	Observations	Reference
	bw/day Gestation day 6-15	Maternal: NOAEL 250 mg/kg bw/day Developmental: 250 mg/kg bw/day	

Two multigeneration studies in rats did not reveal a teratogenic potential or any adverse effects of bentazone on the reproductive performance (Anonymous 1973 (Doc. No 73/010) and Anonymous 1989 (Doc. No. 89/0068)). It is noted that the dose levels in the 3-generation study were quite low. This may also apply to some of the teratogenicity studies.

In Anonymous 1973 (Doc. No. 73/010) no clinical signs were observed and the body weight of the parental animals remained unaffected. 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. Fertility and rearing behaviour of the animals were not affected. The development of the pups was comparable in all the groups. 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) were subjected to histopathological examination. No substance-induced gross-pathological or histopathological changes occurred.

In Anonymous (1989; Doc. No 89/0068) the only pup finding was a decreased mean body weight of F1 and F2 pups in the 800 ppm and 3200 ppm groups. There is no substance-induced effect on fetal body weight at PND1 as shown by unaffected mean litter weight. The 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 18).

Table 18: F1 mean litter weight at PND 1 and 4 [g] (Anonymous (1989; Doc. No. 89/0068))

	Mean values ± SD [g] / (%Deviation to current control) [1]			
PND	**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.

The reduced pup weight at PND4 and 7 at 800 and 3200 ppm is unlikely to be directly caused by the substance or the substance via lactation, but rather a consequence of maternal toxicity (manifested as reduced feed intake during PND1-4). 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 19A). At 800 ppm dams # 159 and #164 lost their entire litters by day 4 post partum. At 3200 ppm dam # 191 lost its entire litter by day 4 post partum. This finding in litter loss was considered not to be related to treatment with the test article. Taking only those dams of the 800 ppm group 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 19A). 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.
Overall pup weight effects are correlated to higher litter size and/or to significant reduced maternal feed intake within the early lactation phase.

Table 19A: 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] maternal	BWG [g]	Mean Pup weight [g]		
	Gestation*						Lactation										
	Days 0-7	Dev. to Control	Days 7-14	Dev. To Control	Days 14-21	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	PND1	PND4
152	21	107%	20	100%	19	94%	25	92%	41	105%	47	92%	218	33	14	5.1	6.6
155	19	97%	21	105%	20	99%	5	18%	33	84%	49	96%	244	-9	13	5.4	4.8
156	22	112%	22	110%	23	114%	19	70%	35	89%	47	92%	253	7	15	4.9	6.3
159	24	122%	22	110%	24	119%	3	11%	-	-	-	-	258	-5	14	5.7	-
164	22	112%	22	110%	23	114%	10	37%	-	-	-	-	279	-26	5	5.5	-
167	21	107%	20	100%	21	104%	14	51%	32	82%	37	73%	263	4	9	5.8	5.5
171	23	117%	23	115%	19	94%	4	15%	28	71%	52	102%	261	-14	13	5.2	5.6
172	20	102%	22	110%	-	-	30	110%	42	107%	55	108%	259	22	15	4.9	6.8
175	17	87%	19	95%	19	94%	18	66%	62	158%	47	92%	224	31	11	5.0	6.0
Mean	21	107%	21.2	106%	21	104%	**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	21.1	108%	21.3	106%	21.3	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%**

* In the RAR of Bentazone the average intake of D0-7, D7-14 and D14-21 are added together. In this table the Dossier Submitter presented the average intake per period during gestation.

In addition to the analysis of the 800 ppm group as presented in the RAR of bentazone, the Dossier Submitter conducted a similar analysis for the 3200 ppm group as well which is presented below (see Table 19B). Taking only those dams of the 3200 ppm group into account which had a total litter loss or whose pups showed reduced pup weights over the period of PND 4-7 which are #182, #185, # 189, #191, and #194, the mean food consumption during lactation period was reduced compared to the control (Table 19B). Between PND 1 and 4 the food consumption was reduced about 63% compared to the control. In contrast to the dams in the 800 ppm group the body weight gain was not reduced between PND 1 and 4 compared to the 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 # 182. This leads to a reduced food consumption of 50.3% of control and the mean maternal body weight gain to 13.5 g between PND1 and 4 (84.3% of control). This demonstrates an more pronounced maternal toxicity at 3200 ppm based on a significantly reduced food intake.

Table 19B: P-generation nursing the F1 pups: 3200 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.

3200 ppm Dam #	Food consumption [g/animal/day]												BW [g]	BWG [g]	Mean Pup weight [g]		
	Gestation						Lactation						maternal				
	Days 0-7	Dev. to Control	Days 7-14	Dev. to control	Days 14-21	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	PND1	PND4
182	21	107%	22	110%	22	109%	31	114	40	102	52	102	248	35	15	4.8	6.2
185	19	97%	18	90%	11	54%	20	73	30	76	40	78	219	18	6	5.0	6.6
189	18	92%	18	90%	20	99%	14	51	36	92	47	92	199	13	13	4.6	4.9
191*	16	82%	17	85%	18	89%	1	4	5	13	19	37	181	-8	11	4.5	-
194	20	102%	21	105%	21	104%	20	73	40	102	52	102	232	31	14	5.0	5.8
Mean	18.8	96%	19.2	96%	18.4	91%	**17.2**	**63%**	**30.2**	**77%**	**42**	**82.2%**	215.8	17.8	11.8	**4.8**	**5.9**
Dev. to control													**87.5%**	106%		**79.7%**	**69%**
Under exclusion of #182 which showed reduced pup weight despite normal food intake																	
Mean	13.8	50.3	27.8	70.8	39.5	77.3	**13.8**	**50.3%**	27.8	70.8%	39.5	77.3%	**207.8**	**13.5**	**11**	**4.8**	**5.8**
Dev. to control													**80.4%**	**84.3%**		**79.7%**	**69%**

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 20). The high dose group showed the peak at day 7 with a -8.8 % reduction vs. control.

Table 20: 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 2011/1145234); Anonymous 2011 (Doc. No. 2011/1248852). 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 21: 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 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.

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 (Table 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.

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 1 for the F1 generation and Figure 2 for the F2 generation below.

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

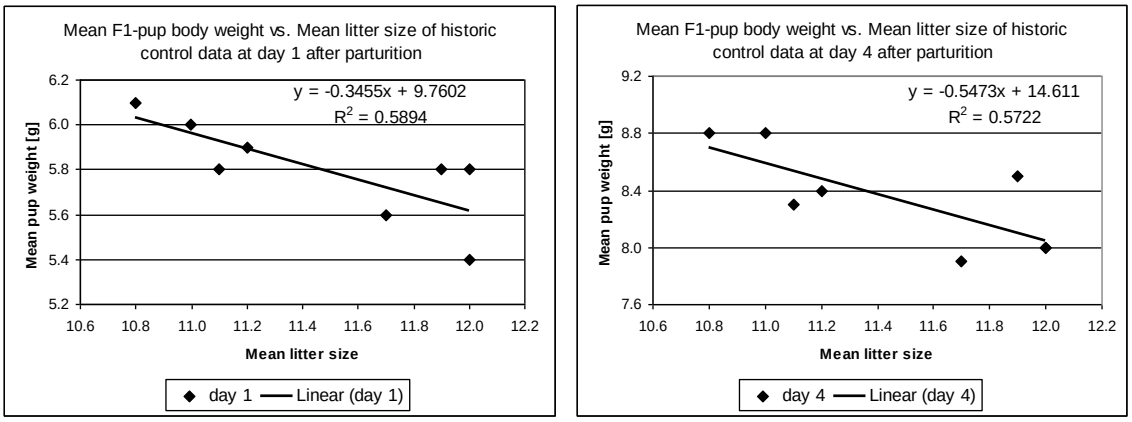
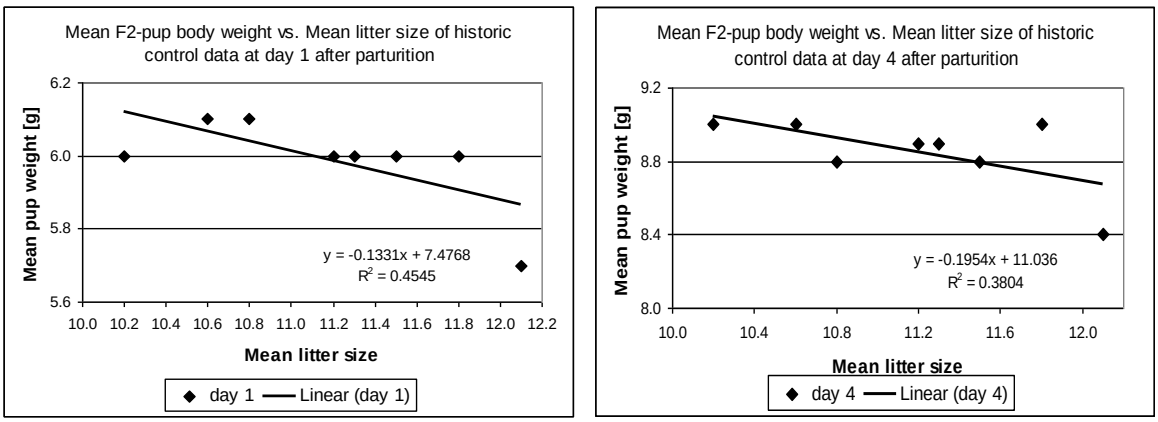


Figure 2: Historical control data: Correlation of mean litter size and mean pup weight at PND 1 and 4 in the F2-pup generation



Developmental study rat – range-finding (1991) (Anonymous 1991, Agrichem file no. R 463)

In a dose-finding study Anonymous 1991 (Agrichem file no. R 463) using 5 dams per dose group there was a dose-dependent increase in post-implantation losses in all treated groups (50; 150 and 450 mg/kg bw/day in bi-destilled water) reaching statistical significance at the two upper dose levels. The corresponding total number of fetuses and of fetuses per dam were decreased in the groups receiving 150 and 450 mg/kg bw/day.

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. 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. However, dead or abnormal fetuses were not noted in any treated group.

Developmental study rat (1991) – by gavage (Anonymous 1991, Agrichem file no. R 22)

The increased post-implantation losses, decreased total number of fetuses and of fetuses per dam, the occurrence of fetal resorptions and a reduced mean fetal weight were not confirmed by another dose-finding study (Anonymous 1991, Agrichem file no. 22). 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 in dams by 8.8% during the first half of the treatment period. The body weight of dams was significantly lower as compared to the control group from day 8 until day 21 p.c. (Table 22). Necropsy of the dams did not reveal any abnormal findings.

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 23).

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.

Table 22 Maternal body weight

Group	**1**	**2**	**3**	**4**	**5**	**6**
Dose (mg/kg bw/day)	**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%)**

Table 23: Fetal body weight

Group	**1**	**2**	**3**	**4**	**5**	**6**

Dose (mg/kg bw/day)	**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

Developmental study rat (1986) – by gavage (Anonymous 1986, Doc. No. 86/421)

Bentazone was administered as 4% aqueous CMC solution to 25 pregnant female Wistar rats per dose group from day 6-15 p.c. by oral gavage at dose levels of 0, 40, 100, and 250 mg/kg bw per day (Anonymous 1986, Doc. No. 86/421). 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 per day, which became apparent between days 6 and 11, was considered test substance-related. However as the effect was only slight (-5.6%) the effect was not considered to be adverse (Table 24).

The investigation of reproduction data revealed a test-substance related, statistically significant increase of the post-implantation loss (number of fetal resorptions) in the dams of the 250 mg/kg bw/day group, with correspondingly reduced number of live fetuses (Table 25). 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. 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 foetuses. This isolated finding was assessed as a consequence of a delayed maturation as indicated by the decreased fetal body weight.

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 fetal NOEL 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.

Table 24: Food consumption and body weight development in rat administered Bentazone during days 6 to 15 of gestation

Dose level (mg/kg bw/day)	**0**	**40**	**100**	**250**
Food consumption (g/animal/day)				
Day 0 to 6 Δ%	19.8	19.9 +0.5	20.2 +2.0	19.9 +0.5
Day 6 to 11 Δ%	21.3	21.1 -0.9	20.6 -3.3	**20.1*** **-5.6**
Day 11 to 16 Δ%	22.5	21.9 -2.7	22.1 -1.8	21.8 -3.1
Day 16 to 21 Δ%	23.0	22.3 -3.0	22.5 -2.2	22.1 -3.9
Body weight gain (g)				

Day 0 to 6 Δ%	22 10.9	21 10.4	25 12.8	23 11.4
Day 6 to 11 Δ%	19 8.5	18 8.1	15 6.8	16 7.1
Day 11 to 16 Δ%	51 19	49 18.6	51 19.5	43 16.2
Day 16 to 21 Δ%	96 43.0	90 40.5	92 41.6	84 37.5

* $p<0.05$

Table 25: Total number of fetal resorptions, mean number of living fetuses per litter and mean fetal weight

Dose level (mg/kg bw/day)	**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

Developmental study rat (1982) – by diet (Anonymous 1982, Doc. No. 84/066)

Furthermore, effects on the development of the skeleton, such as reduced/altered ossification, were seen in Anonymous 1984 (Doc. No. 84/066). The study is considered supplementary only since the route of administration (diet) and duration of treatment (post-coitum day 0-21) does not allow a comparison with other studies on teratogenicity. However, it provides important additional information.

Clinical symptoms noted were alopecia in one animal of the 8000 ppm (about 631 mg/kg bw/day) 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. Food consumption was increased in the 2000 ppm (about 162 mg/kg bw/day) and 4000 ppm (about 324 mg/kg bw/day) groups, whereas a significant decreases was noted in the 8000 ppm group. Water consumption in the 4000 ppm and 8000 ppm groups was significantly higher than in the control group. Amniotic fluid weight was increased in groups receiving 4000 ppm or 8000 ppm. 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.

There were no significant differences between the number of implantations, embryo 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.

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.

Developmental study rat (1971) – study supplementary only (Anonymous 1971, Doc. No. 71/0041)

Either increased implantation loss and number of runts or reduced foetal weights in rat was seen in Anonymous 1971 (Doc. No. 71/0041). Also increased incidences of anasarca were seen.
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/day, the resorption rate was drastically increased (Table 26). In addition, the fetuses showed a decrease in body weight, an increase in the number of runts and an increase in the frequency of anasarca. The occurrence of anasarca was confined to this group. 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.

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.

Table 26: Resorption rate and number of runts

Dose level (mg/kg bw/day)	**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)

Developmental study rat (1978) – study supplementary only (Anonymous 1978 (Doc. No. 78/039)

The embryo-/fetotoxic effects as observed in Anonymous 1971 (Doc. No. 71/0041) 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 (Anonymous 1978, Doc. No. 78/039). 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.

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.

Developmental study rat (El-Mahdi MM and Lofti MM 1988) – study supplementary only

The effects seen in the teratogenicity studies with bentazone were also seen after single doses of Basagran (bentazone preparation) given by gavage to the rat at different times during gestation in the published study of El-Mahdi and Loftie (1988).

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 classification purposes. However, it provides supplementary information since time-dependence of fetal effects was investigated.

Developmental study rabit (Anonymous 1987, Doc. No. 87/058)

16 pregnant Chinchilla rabbits per group were administered 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.

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 (Table 27) (Anonymous 1987, Doc. No. 87/058). 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 (Table 28). During the skeletal investigations, isolated findings were noted in all groups, including the control (Table 29). There were no signs of test substance-relationship. No substance-induced teratogenicity was observed.

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.

Table 27: 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
Cesarean 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

Table 27: Caesarean section data

Dose level [mg/kg bw/day]	**0**	**75**	**150**	**375**
- 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[+])
- 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

Table 28: 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 29: 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

Table 29: Incidence of skeletal malformations and variations

Dose level [mg/kg bw/day]	0	75	150	375
Total skeletal abnormal findings - 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

Developmental study rabit (1984)- supplementary only (Anonymous 1984, Doc. No. 84/048)

Bentazone was administered as 0.5 % aqueous CMC solution by oral gavage to groups of 15 female Himalayan ChBB:HM rabbits per dose group on days 6 - 18 of gestation at dose levels of 0, 50, 100 and 150 mg/kg bw per day (Anonymous 1984, Doc. No. 84/048). No developmental toxicity and teratogenic effects were observed in Himalayan ChBB:HM rabbits. Neither clinical signs of toxicity nor any adverse effect on body weight and body weight gain were noted at any dose level (0; 50; 100 and 150 mg/kg bw/day). 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/day fetuses were substantially similar to actual control values (Table 30).

Under the conditions of this study, the NOAEL for both maternal toxicity and embryo-/fetotoxicity was 150 mg/kg bw/day. No teratogenic effects were found.

Table 30: Incidence of skeletal malformations and variations

Dose level [mg/kg bw/day]	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

Table 30: Incidence of skeletal malformations and variations

Dose level [mg/kg bw/day]	**0***	**0****	**50**	**100**	**150**
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

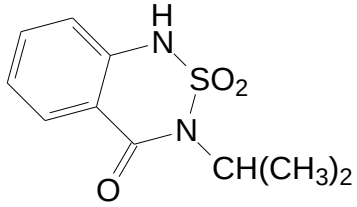
* control group untreated
** control group treated with CMC

10.10.5 Supplementary repeated dose studies

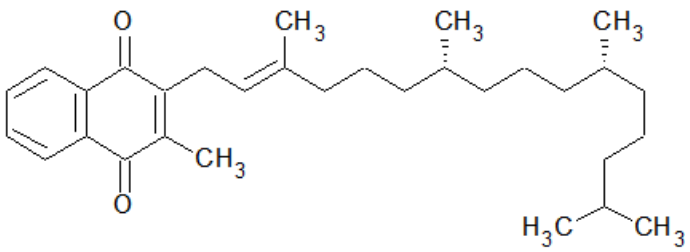
Supplementary long term carcinogenicity studies and repeated dose toxicity studies to indicate the type of maternal toxicity which can be expected at certain doses are included in section 10.9 and 10.12 respectively.

10.10.6 Mechanism of action

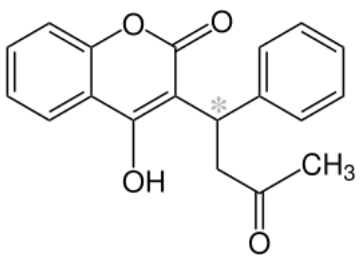
Besides body weight change which was affected in all species at high dose levels, repeated dose studies further show that a prolonged blood coagulation time and a decreased hematocrit and hemoglobin content were the most frequent findings observed (section 10.9 and 10.12). As discussed at the EFSA expert meeting the prolonged coagulation time caused by Bentazone which can results in hemorrhages in the rat, mouse and dog, has structural resemblance to vitamin K and anticoagulants as warfarin (Figure 3).



Bentazone



Vitamine K



Warfarin

Figure 3: Structural formula of Bentazone, vitamin K and warfarin

Warfarin gives similar developmental effects that are seen in the studies with bentazone, like post-implantation loss, reduced foetal weights and reduced/altered ossification (Mirkova, E.; Antov, G., 1983; Howe, A.M.; Webster, W.S., 1992; Kubaszky, 2009; RAC opinion on the classification of warfarin, 2014). However, the post-implantation losses were not affected at any of the dose levels or treatment regimens in surviving dams (RAC opinion on the classification of warfarin, 2014). Developmental studies with the anticoagulant flocoumafen did not show post-implantation loss (RAC opinion on the classification of flocoumafen, 2014). The observed increase in post-implantation loss with bentazone is not considered secondary to the potential decrease in blood coagulation and increase in haemorrhaging.

10.10.7 Short summary and overall relevance of the provided information on adverse effects on development

Two multigeneration studies in rats did not reveal a teratogenic potential or any adverse effects of bentazone on the reproductive performance. The only pup finding, in the two-generation study carried out on rats, was a decreased mean body weight of F1 and F2 pups in the 800 ppm and 3200 ppm groups. There is no substance-induced effect on fetal body weigth on PND1. The reduced pup weight at PND4 and 7 at 800 and 3200 ppm is unlikely to be directly caused by the substance, 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.

The developmental toxicity studies revealed an effect on post-implantation loss. This was observed primarily in rat. See table 32 for a summary of the effects on post-implantation loss in the rat developmental studies. In a dose-finding study (Anonymous 1991 (Agrichem file no. R 463)) and in two developmental studies with bentazone, increased implantation loss was observed in the rat, see table 25 (Anonymous 1986 (Doc. No. 86/421)) and Table 26 (Anonymous 1971 (Doc. No. 71/0041)). However, the elevated resorption rate seen in Anonymous 1971 (Doc. No. 71/0041) were not reproducible when the study was repeated six years later on the same rat strain under the same conditions showing no effects up to 200 mg/kg bw/day in Anonymous 1978 (Doc. No. 78/039). The post-implantation loss was not observed in a developmental study with administration by diet and longer duration of treatment. Increased implantation loss was seen in one high dose rabbit study (dose 375 mg/kg bw/day; 125 implantations and 11 losses) (Anonymous 1987 (Doc. No. 87/058).

Table 32: Summary table of effects seen on post-implantation loss in rat developmental toxicity studies

Study	**Strain and number of animals**	**Exposure period (gestation days)**	**Route**	**dose (mg/kg bw/day)**	**Post-implantation loss**	**Maternal toxicity**
Anonymous 1991; Agrichem file no. R463	Wistar rat (n=5)	6-15	Oral, via gavage	450	Increased	No effect
				150	Increased	No effect
				50	Not increased	No effect
Anonymous 1991; Doc. No. Agrichem file no. R22	Wistar rat (n=25)	6-15	Oral, via gavage	360	Not increased	No effect
Anonymous 1986; Doc.	Wistar rat	6-15	Oral, via	250	Increased	No effect

No. 86/421	(n=25)		gavage			
				100	Not increased	No effect
				40	Not increased	No effect
Anonymous 1982; Doc. No. 84/066	Rat of the SD/CRJ strain (n=21-23)	0-21	Oral, via diet	631 (8000 ppm)	Not increased	Reduced bw gain, hematuria, amniotic fluid and water consumption increased
				324 (4000 ppm)	Not increased	No effect
				162 (2000 ppm)	Not increased	No effect
Anonymous 1971; Doc. No. 71/0041	Sprague-Dawley rat (n=20-32)	6-15	Oral, via gavage	200	Increased	No effect
				66.7	Not increased	No effect
				22.2	Not increased	No effect
Anonymous 1978; Doc. No. 78/039	Sprague-Dawley rat (n=26-29)	6-15	Oral, via gavage	200	Not increased	No effect
				66.7	Not increased	No effect
				22.2	Not increased	No effect
El-Mahdi MM and Lofti MM 1988	Rat (strain not specified)	Single dose on day 6, 8, 11,14, 16	Oral, via gavage	12.0	Increased	No details given
				43.2	Increased	No details given
				96	Increased	No details given

Either increased number of runts or reduced foetal weights were seen in the rat developmental study of Anonymous 1971 (Doc. No. 71/0041) (table 26) and reduced foetal weights were seen in the study of Anonymous 1986 (Doc. No. 86/421) (Table 25). Furthermore, effects on the development of the skeleton, such as reduced/altered ossification, were seen in these rat studies (Anonymous 1984 (Doc. No. 84/066), Anonymous 1986 (Doc. No. 86/421). Increased incidences of anasarca were also seen in one study (Anonymous 1971 (Doc. No. 71/0041). The effects seen in the teratogenicity studies with bentazone were also seen after single doses of Basagran (bentazone preparation) given to the rat by gavage at different times in gestation in the published study of El-Mahdi and Loftie (1988). 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.

In rat developmental studies increased post-implantation loss was seen at doses with no maternal effects. Other foetal effects (increased number of runts, reduced foetal weights and reduced/altered ossification, increased incidences of anasarca) were observed at doses with only slight maternal effects such as reduction in food consumption and body weight gain. Body weight changes were observed in rat at high dose levels (1600 ppm and 200 mg/kg bw/day) during repeated dose studies (Sections 10.9 and 10.12). The reduced maternal food intake at the highest dose cannot explain the decreased pup body weight and related delay in

ossification nor the increase in foetal resorptions seen at this dose level Anonynmous 1986 (Doc. No. 86/421). However, it is noted that general toxicity findings were observed in other repeated dose and long term toxicity studies that are normally not investigated in the developmental toxicity studies. These other effects with rats included kidney toxicity and changes in haematological (coagulation time) and clinical chemistry parameters. According to the CLP criteria a consistent relationship between general maternal toxicity and developmental effects is not established. A possibility for a direct effect on the foetus thus cannot be ruled out.

There is no information on epidemiological studies and case reports in humans on reproductive toxicity.

There are no indication from the toxicity studies that bentazone has any endocrine effect. In the two multigeneration studies in rats and in in vitro studies from published literature on some endocrine endpoints, no effects on fertility were observed. It was shown that bentazone did not have an estrogenic effect in a yeast screen (YES-assay) or the E-screen assay, had no estrogenic/androgenic effect in an in vitro Luciferase reporter assay and has no binding affinity to the human androgen receptor.

10.10.8 Comparison with the CLP criteria

No information on the effects of bentazone on pregnant women is available. Therefore, classification in category 1A is not applicable.

Classification in category 1B or category 2 is based on evidence in animal studies. No clear effects on development were observed in the studies in rabbits. In the studies in rats, some effects were observed such as reduced fetal body weight and increases in some variations that are not considered as leading to classification. An increased number of runts and an increase in anasarca was reported in a supplemental study. However, this effect was not observed in a range of additional studies in rats and therefore considered not related to exposure. The most important effect noted in the available developmental studies in rat is an increase in post-implantation loss resulting in a decrease in the number of live foetuses. This effect is observed in some but not all studies. No explanation can be provided for this inconsistency. Maternal toxicity at the dose levels inducing an increased post-implantation loss (range 150-205 mg/kg bw/day) was not observed. Comparison of the relevant dose range with the most comparable study in rats (28-day repeated dose) indicates that no effects were observed at 200 mg/kg bw/day and hemorrhages in kidneys and ovaries were observed at 554 mg/kg bw/day (Anonymous 1981 (Doc. No. 81/10240). Therefore, it cannot be excluded that the increase in post-implantation loss is related to the increase in maternal hemorrhages. However, other substances which induce a reduction in blood coagulation and increase in hemorrhaging as described above do not increase post-implantation loss. Further, there is no specific information available regarding the mechanism by which bentazone induces an increase in post-implantation loss. As the non-relevancy of this effect for humans is not proven,it is considered that the observed effect is also relevant to humans. Classification in category 1B is applicable when there is clear evidence for developmental effects in the absence of maternal toxicity or considered not to be a secondary non-specific effect of maternal toxicity. Classification in category 2 is applicable when the information is less clear and/or the effects are observed in the presence of maternal toxicity and it is unclear whether the observed fetal effects are a secondary non-specific effect of the maternal toxicity. As the increase in post-implantation loss was observed in some studies but not in other studies, the information concerning this effect could be considered less than clear. No maternal toxicity was observed at dose levels inducing post-implantation loss. However, the maternal effects that were not determined in the developmental studies but observed in repeated dose studies at comparable dose levels such as effects on coagulation are not known to induce post-implantation loss. Therefore, the observed post-implantation loss is not considered secondary to maternal toxicity. Overall, classification is considered borderline between category 2 and 1B because the increase in post-implantation loss is not observed consistently and this difference cannot be explained. Classification in category 2 is proposed because the foetal effects (post-implantation loss) are not consistent. It was increased in dose finding study (Anonymous1991; Agrichem file no. R463) but not in the main study (Anonymous 1991; Agrichem file no. R22). It was increased in a supplemental study (Anonymous 1971 (Doc. No. 71/0041) but not when the study was repeated six years later on the same rat strain under same conditions (Anonymous 1978 (Doc. No. 78/039).

10.10.9 Adverse effects on or via lactation

Not evaluated in this dossier.

10.10.10 Comparison with the CLP criteria

Not evaluated in this dossier.

10.10.11 Conclusion on classification and labelling for reproductive toxicity

Bentazone should be classified as Repr. 2 (H361d: Suspected of damaging the unborn child).

10.11 Specific target organ toxicity-single exposure

No evaluated in this dossier.

10.12 Specific target organ toxicity-repeated exposure

No evaluated in this dossier. However, a summary is provided to support the evaluation of the endpoint reproductive toxicity. The repeated dose toxicity studies are included to indicate the type of other maternal toxicity which can be expected at certain doses, see Table 33. For description of the studies mentioned in the Table 33, see Annex I. Repeated dose studies show that in addition to body weight change which was affected in all species at high dose levels, a prolonged blood coagulation time and a decreased hematocrit and hemoglobin content were the most frequent findings observed. The comparison of the NOELs has shown that interspecies variation of bentazone is rather low.

Table 33: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
ORAL			
No specific guideline GLP: No Rat, Fisher 344/CRJ, both sexes 8/sex/dose	Bentazone (purity: 93.9%; batch no. not available) Oral (via diet) 0; 600; 1800; 5000 and 10000 ppm; equivalent to: 0, 64/71, 196/217, 554/607, 1068/1132 (m/f) mg/kg bw/day Subacute study (31-33 day)	10000 ppm: mortality; reduced bw and bw gain; changed hematological parameters; hemorrhages in various tissues; increased weight of liver and kidneys; decreased weight of heart and testicles. 5000 ppm: hemorrhages in kidneys and ovaries. NOAEL 1800 ppm LOAEL 5000 ppm	Anonymous 1981 (Doc. No. 81/10240)[a] Klimisch score: 4

No specific guide line. GLP: No Mouse, B6C3F1/CRJ mice, both sexes 6/sex/dose	Bentazone technical (purity not available; batch no. not available) Oral (via diet) 0; 400; 2000; 5000 and 10000 ppm; equivalent to: 0, 91/100, 407/487, 905/1004, 1469/1663 (m/f) mg/kg bw/day Subacute study (30 day)	5000 ppm and 10000 ppm: up to 100% mortality; hemorrhages in various organs. 2000 ppm: changed coagulation parameters. NOAEL not obtained LOAEL 2000 ppm	Anonymous, 1981 (Doc. No. 81/10239)[a] Klimisch score: 4
No specific guideline. GLP: No Rat, SPF breed Sprague-Dawley, both sexes 20/sex/dose	Bentazone technical ((purity not available; batch no. not available) Oral (via diet) 0; 70; 200; 800 and 1600 ppm; equivalent to: 3.5; 10; 40; 80 mg/kg bw/day 90 days	1600 ppm: retarded bw gain; increased relative kidney weight. 800 ppm: increased relative kidney weight in females. NOAEL 200 ppm (about 10 mg/kg bw/day) LOAEL 800 ppm	Anonymous 1970 (Doc. No. 70/008)[a] Klimisch score: 3
OECD 408 GLP: yes Rat, Wistar, both sexes. 10/sex/dose	Bentazone technical (Batch no. N 187; purity 97.8%) Oral (via diet) 0; 400; 1200 and 3600 ppm; equivalent to: 0; 25.3/28.9; 77.8/86.1 and 243.3/258.3 (m/f) mg/kg bw/day 90 days	3600 ppm: mortality (4/10 males); retarded bw gain in females; changed hematological and clinicochemical parameters. 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 NOAEL 1200 ppm LOAEL 3600 ppm	Anonymous 1987 (Doc. No. 87/0173)[a] Klimisch score: 1

OECD 408 GLP: yes Rat, Crl:WI, both sexes.	Bemtazone, batch no. COD-001416, purity 100%. Oral (via diet) 3600 ppm, equivalent to: 238/252 (m/f) mg/kg bw/day 90 days Bentazone-Na, batch no. COD-001417, purity 91.9%. Oral (via diet) 0, 475, 1425, 4275 ppm; equivalent to: 0, 31/42, 91/98 and 290/304 (m/f) mg/kg bw/day 90 days	3600 ppm Bentazone: increased kidney (abs. & rel.) and liver (rel.) weights in females; changed haematological parameters in males; changed clinicochemical and urinary parameters NOAEL (Bentazone) <3600 ppm LOAEL (Bentazone) 3600 ppm 4275 ppm Bentazone-Na: increased kidney (abs. & rel.) and liver (rel.) weight in females; changed haematological parameters in males; change clinicochemical parameters. 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. NOAEL (Bentazone-Na): 1425 ppm LOAEL (Bentazone-Na) 4275 ppm	Anonymous 2011 (Doc No: 2011/1173365) [a] Anonymous 2012 (Doc No. 2012/1009658) Klimisch score: 1
No test guideline GLP: no Dog, Beagle, both sexes 3/sex/dose	Bentazone technical (purity not available; batch no. not available) Oral (via diet) 0; 100; 300; 1000 and 3000 ppm; equivalent to: 0; 4.0; 12.0; 39.6 and 113.8 mg/kg bw/day for both sexes. 90 days	3000 ppm: mortality (3/6 animals); sedation; hemorrhage conjunctivitis; ulcerative stomatitis (males); changed hematological and clinicochemical parameters; increased relative and absolute liver and kidney weights. 1000 ppm: sedation (1/6 animals). NOAEL 300 ppm LOAEL 1000 ppm	Anonymous 1970 (Doc. No. 70/009) [a] Klimisch score: 2
OECD 452 GLP: yes Dog, Beagle, both sexes 6/sex/dose	Bentazone technical (purity: 97.8%, batch no. N 187) Oral (via diet) 0; 100; 400 and 1600 ppm; equivalent to: 0, 3.04/3.29, 13.07/13.2 and 49.72/54.83 (m/f) mg/kg bw/day 1 year	1600 ppm: transient decrease in bw; changes in hematological parameters. NOAEL 400 ppm LOAEL 1600 ppm	Anonymous 1989 (Doc. No. 89/0049) [a] Klimisch score: 1
DERMAL			

OECD 410 GLP: yes Rabbit, SPF New Zealand White, both sexes 5/sex/dose	Bentazone (purity: 97.64%, batch no. N 194). Administered dermally for 21 days at dose levels of 0 (solvent control); 250; 500 and 1000 mg/kg bw.	No systemic or local toxicity. NOAEL 1000 mg/kg bw/day	Anonymous 1993 (Doc. No. 93/10760)[a] Klimisch score: 1
No specific guideline. GLP: No Rabbit, New Zealand White, both sexes 6/sex/dose	Bentazone technical (purity not reported; batch no. not available) Administered dermally for 21 days at dose levels of 0; 250; 500 and 1000 mg/kg bw.	No systemic or local toxicity. NOAEL 1000 mg/kg bw/day	Anonymous 1971 (Doc. No. 71/005)[a] Klimisch score: 4

[a] As summarised in the Final addendum to the Renewal Assessment Report for Bentazone, January 2015.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Subacute and subchronic feeding studies were carried out in rats, mice and dogs. In addition to a decrease in body weight which was seen in all species at high dose levels, a prolonged blood coagulation time, diminished hematocrit and reduced hemoglobin were the most frequent findings in these studies. Apart from blood, liver and kidney were identified to be additional target organs. However, the effects observed in these organs were of minor importance. The NOEL in a 4-week oral study in rats was approximately 200 mg/kg bw/day while a NOEL for the subacute mouse study could not be established. In the most recent 90-day rat study, a NOAEL of about 77.8 (males) or 86.1 (females) mg/kg bw/day was obtained confirming previous results. In the 90-day study in dogs, the NOEL was approximately 12 mg/kg bw/day with mortalities and signs of severe intoxication at the highest dose level of 3000 ppm (about 114 mg/kg bw/day) suggesting that the maximal tolerated dose (MTD) was exceeded. In a more recent one-year study in dogs, a similar NOEL of about 13.1 mg/kg bw/day was established.

Short-term dermal administration of bentazone up to 1000 mg/kg bw did not cause any adverse effects in rabbits. The comparison of Bentazone-sodium and Bentazone-acid in Wistar rats over a period of 3 months after oral administration revealed similar signs of toxicity for both compounds at the high equimolar dose.

The comparison of Bentazone-sodium and Bentazone-acid in Wistar rats over a period of 3 months after oral administration revealed similar signs of toxicity for both compounds at the high equimolar dose. Furthermore, the findings were well comparable with the findings of the former subchronic study, although the no observed effect levels seem to be different, with Bentazone-acid showing effects at 1200 ppm versus no effect on clinicochemical parameters seen at 1425 ppm with Bentazone-sodium. These differences are considered to reflect the biological variation within the animals.

10.12.2 Comparison with the CLP criteria

No evaluated in this dossier. However, a summary is provided to support the evaluation of the endpoint reproductive toxicity.

10.12.3 Conclusion on classification for specific target organ toxicity – repeated exposure

Not evaluated in this dossier.

10.13 Aspiration hazard

Not evaluated in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

The environmental hazards of the herbicide bentazone were assessed in the Renewal Assessment Report (RAR), and it addenda (January 2015) concerning the renewal of the approval of the active substance bentazone. The RAR is publicly available via the EFSA web site (http://dar.efsa.europa.eu/dar-web/provision). The conclusion document on the peer review of the pesticide risk assessment of the active substance bentazone (20th April 2015) is also publically available on the EFSA web site (https://www.efsa.europa.eu/en/efsajournal/pub/4077) (EFSA, 2015). Where available endpoints for bentazone were taken from the RAR and the EFSA conclusion document on bentazone. Studies considered acceptable in the RAR (reliability score of 1 or 2) have been included in this report and were considered for classification purposes. The study summaries as presented in the RAR are included in Annex 1. All studies were carried out under GLP unless indicated otherwise. Studies were carried out in accordance with relevant test guidelines. Minor deviations were noted in some cases but these did not affect the overall reliability of the studies. The deviations are included in the summaries where relevant.

Additional sources were consulted for data. No data were found on ECHA's public dissemination site, as bentazone is not registered under REACH, i.e. the substance is only pre-registered (envisaged deadline was 30-11-2010). Publically available data were obtained by searching several databases including e-chemportal, PubMed, ToxNet, EPA's ECOTOX knowledgebase and publications such as the JRC draft document on bentazone (v2.2) from 11th February 2015 (JRC, 2015), the BASF comments on the JRC draft document (Maier et al., 2014) and RIVM report 2015-0095 that investigated leaching of bentazone to groundwater (van der Linden et al., 2015). Searching EPA's ECOTOX knowledgebase using bentazone's CAS number yielded 280 effect concentrations for the aquatic environment (accessed April 5th 2019). Public literature data were only included in this dossier when considered relevant. Endpoints from databases were only used for classification purposes when original test reports and/or robust study summaries could be assessed for their reliability. QSAR estimates have only been added to complement the dataset if reliable experimental data were lacking (e.g. biodegradability).

11.1 Rapid degradability of organic substances

Table 10: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Biodegradation screening study Method: non-guideline; non-GLP; CO_2-evolution in water sediment system; aerobic; technical bentazone (purity 97.5%)	Degradation after 117 days at 20 °C based on: CO_2 production: 27.9-59.7% at 10 mg/L 0-0.5% at 20 mg/L Residuals: 0% at 10 and 20 mg/L	Considered supplemental in RAR. Not a ready biodegradability study (sediment instead of activated sludge; exposure 117 instead of 28 days). Results based on CO_2-production are unreliable (Klimisch score of 3). Results based on residual measurements are reliable with restriction (Klimisch score of 2). **Supportive data**	van der Hoek & Kreuk, 1987[a]
Ready biodegradability Method: BIOWIN v4.10	Not readily biodegradable BIOWIN 3: weeks-months BIOWIN 5 and 6: not readily biodegradable	QSAR Results based on BIOWIN 1, 2, 3, and 4 are unreliable (Klimisch score of 3).	EPA, 2012

Method	Results	Remarks	Reference
		Results based on BIOWIN 5,6 and 7 are reliable with restriction (Klimisch score of 2) **Supportive data**	
Hydrolysis Method: statement in RAR	At 25 °C bentazone is hydrolytically stable at pH 5, 7 and 9.	Accepted in RAR. Reliable with restrictions Klimisch score of 2	Eswein & Panek, 1986a[a]
Phototransformation in water Method: OECD TG 316; GLP; ^{14}C-bentazone (radiochemical purity 97.5%)	artificial sunlight (618 W/m^2) DT50: 3, 5.4 and 3.9 days at pH 5, 7 and 9, resp. (22 °C). dark stable at pH 5,7 and 9 (22 °C)	Accepted in RAR. Direct photolytic degradation in water occurs. Dark samples showed no hydrolysis. Klimisch score of 1	Singh, 2011a[a]
Phototransformation in water Method: non-guideline; non-GLP; bentazone (BAS 351H), (purity 99.7%)	Natural sunlight (summer) DT50: 2.17, 4.08 and 5.12 days in canal water, lagoon (=brackish) water, and ultra-pure water (temperature not reported) dark stable in ultra-pure water (temperature not reported)	Considered additional in RAR. Direct photolytic degradation in water occurs. Dark samples showed no hydrolysis. Klimisch score of 1	Housari et al., 2010a[a]
Phototransformation in water Method: non-guideline; GLP; bentazone (purity: 99%; radiochemical purity: 99.3%)	artificial sunlight (456 W/m^2) DT50: 47.8 hours at pH 7 (20 °C).	Accepted in RAR. Direct photolytic degradation in water occurs. Klimisch score of 1	Dawson, Lynn & McCorquodale, 2003[a]
Half-life in air Method: AOPWIN v1.92a	hydroxyl radicals: 2.06 hours	QSAR	EPA, 2012
Water/soil degradation EPA Guidelines § 162-4 (aerobe) and 162-3 (anaerobe); non-GLP; purity not reported;	DT50 not reported; aerobic 3.1-4.5% mineralization after 374 days at 25°C Mostly bentazone, minor levels of transformation products detected. anaerobic Practically no mineralization after 365 days at 25°C. At test end, bentazone amounted to 87.7 and 5.5% in water and soil, respectively.	Considered additional in RAR. Soil instead if sediment. Unclear what activation of soil encompasses. Hardly any mineralization. Klimisch score of 2 **Supportive data**	Gerhardt and Hamm, 1987[a]
Water/sediment	Degradation	Accepted in RAR.	Bieber, 1994;

Method	Results	Remarks	Reference
degradation BBA guideline IV, 5-1; non-GLP; ^{14}C-bentazone; aerobe purity not reported;	DT50$_{, \text{total system}}$: 688 and 940 days at 20 °C. <u>Dissipation</u> DT50$_{, \text{water}}$: 701 and 678 days; DT50$_{, \text{sediment}}$: 595 and 568 days at 20 °C. Mineralization 2.6% after 100 days at 20 °C.	Klimisch score of 1 **Supportive data**	Timme and Frehse, 1980; Timme et al., 1986; Matejek, 2012a [a]
Water/sediment degradation non-guideline non-GLP; ^{14}C-bentazone; aerobe; radiochemical purity 99.5-100%	DT50 could not be determined Mineralization 1.3% after 106 days (temperature not reported)	Accepted in RAR. Klimisch score of 2 **Supportive data**	De Vries, 1996[a]
Soil degradation studies lysimeter guideline according to SETAC (1995); non-GLP; aerobic; ^{14}C-bentazone; purity 99.6%	DT_{50} = 15.9 days at 20 °C (= dissipation) DT_{50} = 610 days at 20 °C (= degradation; NER attributed to parent)	Accepted in RAR (DT50 = dissipation). Lysimeter study is not a soil simulation study. Degradation DT50 calculated incl. NER as parent. Klimisch score of 2 **Supportive data**	Ebert, 1995[a] Budde, 2014a[a]
Soil degradation studies OECD TG 307e; GLP; aerobic; ^{14}C-bentazone; purity 96.5%	DT_{50} = 45.1 days at 20 °C (= dissipation) DT_{50} = 352 days at 20 °C (= degradation; NER attributed to parent)	Accepted in RAR (DT50 = dissipation). Degradation DT50 calculated incl. NER as parent. Klimisch score of 2 **Supportive data**	Staudenmaier and Kuhnke, 2010b[a]
Soil degradation studies OECD TG 307e; GLP; (an)aerobic; ^{14}C-bentazone; purity 99.8%	<u>aerobe conditions</u> DT_{50} = 18.5 days at 20 °C (= dissipation) <u>anaerobe conditions</u> DT_{50} = >1000 days at 20 °C (= dissipation)	Accepted in RAR (DT50 = dissipation). Klimisch score of 2 **Supportive data**	Ebert, 2010[a]
Soil degradation studies OECD TG 307e; GLP; aerobic; ^{14}C-bentazone; purity 99.1%	DT_{50} = 30.9, 33.0, 43.4, and 49.1 days at 20 °C (= dissipation)	Accepted in RAR (DT50 = dissipation). Klimisch score of 2	Tornisielo and Sacchi, 2011b[a]

[a] As summarised in the Final addendum to the Renewal Assessment Report for Bentazone, January 2015.
[b] As summarised in (TOXNET 2019), accessed May 2019.

11.1.1 Ready biodegradability

An experimental ready biodegradability study with bentazone is not available. In the RAR reference is made to a non-standard biodegradation screening study conducted with technical bentazone (purity of 97.5%)

using ditch-water and ditch-sediment for 117 days (van der Hoek & Kreuk, 1987). In the RAR it was concluded that the study should be considered supplementary as it is neither a guideline study for ready biodegradability, though reference to OECD TG 301D has been made, nor a water sediment study according to OECD TG 308. Indeed the study setup of this CO_2 evolution study deviates substantially from OECD TG 301B, and as such cannot be used to determine if bentazone is readily biodegradable. Most critical deviations are the use of sediment as inoculum instead of activated sludge and the prolonged exposure of 117 days instead of 28 days. Furthermore, the results are unequivocal, i.e. based on CO_2 production it appears that after prolonged exposure bentazone is degraded in the 10 mg/L treatment (28-60% after 117 days), but not in the 20 mg/L treatment (0.5%). The residual measurements show no degradation (0%) after 117 days, with the initial measurements being slightly higher than those at test end. Thus, it appears that the CO_2 production is the result of other degradation processes in the sediment, rather than bentazone degradation. The Dossier Submitter considers the results based on CO_2 production unreliable (Klimisch score of 3), and the residual measurements reliable with restrictions (Klimisch score of 2). Overall, the dossier submitter considers the **residual based degradation data as supportive for the low biodegradation potential of bentazone**.

The BIOWIN v4.10 QSAR contained within EPI Suite™ version 4.11 (EPA 2012b) consists of six models. Bentazone is predicted to biodegrade fast using a linear biodegradation model (BIOWIN 1) and slow using a non-linear biodegradation model (BIOWIN 2). Ultimate biodegradation, i.e., conversion of bentazone to carbon dioxide (BIOWIN 3), is predicted to occur within weeks to months, while initial steps of biodegradation (BIOWIN 4) are predicted to occur within days to weeks. In two of the models, BIOWIN 5 and 6, representing MITI testing, bentazone was not considered to be readily biodegradable based on microbial oxygen uptake in the OECD 301C test. Bentazone is not predicted to biodegrade quickly under anaerobic conditions (BIOWIN 7). The BIOWIN models estimate the probability of rapid aerobic/anaerobic microbial biodegradation of organic compounds based on the presence of fragments contained within the structure and/or molecular weight. The applicability domains of the models are not explicitly defined, but the manual indicates that care should be taken interpreting the results when: (#1) the molecular weight of the compound falls outside the molecular weight range of the training set compounds; (#2) when the substance has more instances of a given fragment than the maximum for all training set compounds; (#3) when the compound has functional group(s) or other structural features that are not represented in the training set, and for which no fragment coefficient was developed; (#4) when a compound has none of the fragments in the model's fragment library, in which case the predictions are based on molecular weight alone. Bentazone has a molecular weight of 240.3 g/mol and falls within the range of molecular weights of training set compounds (narrowest range: 53 – 697 g/mol). However, Bentazone contains none of the 36 fragments specified in BIOWIN models 1 up to 4, making the estimations of these four models unreliable (Klimisch score of 3). Three fragments were identified by BIOWIN models 5, 6 and 7, but functional groups containing nitrogen and sulphur were not included, making these estimations less reliable (Klimisch score of 2). Thus, **BIOWIN estimates bentazone to be not readily biodegradable.**

Above data are used as supportive for the low biodegradation potential of bentazone.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Bentazone contains no functional groups that can hydrolyse such as esters, amides or epoxides. In the RAR, it is stated that bentazone is hydrolytically stable at 25 °C at pH 5, 7 and 9. Reference is made to an unpublished study report (Eswein & Panek, 1986). The Hazardous Substances Data Bank (HSDB) reports that bentazone is very resistant to hydrolysis referring to Hartley and Kidd (1983) who observed no degradation after 48 hours in 0.1 N sodium hydroxide and in 0.1 N hydrochloric acid (TOXNET, 2019). The aqueous photolysis studies available in the RAR also investigated the stability of bentazone under dark conditions, and did not observe hydrolysis in buffered solutions with pH 5, 7 and 9 during 15 days (Singh, 2011a), nor in ultra-pure water during 8 days (Housari et al., 2010a). **Bentazone is considered hydrolytically stable.**

11.1.4 Other convincing scientific evidence

No data available.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

There are field studies available in RAR. These studies are not considered relevant for classification purposes, and are not further discussed. Field studies have been included in Annex I.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

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

11.1.4.3.1 Biodegradation in water

No data available.

11.1.4.3.2 Biodegradation in water/sediment systems

The RAR contains several studies that investigated degradation of bentazone in water/sediment systems under laboratory conditions and field conditions. The field studies are included in Annex I, but will not be discussed here as they are not considered relevant for classification purposes.

Gerhardt and Hamm (1987) examined aquatic metabolism of ^{14}C-bentazone (purity not reported) under aerobe and anaerobe conditions according to EPA Guidelines § 162-4 and 162-3, respectively. OECD TG 308 is based amongst others on EPA Guideline §162-3. The study was considered additional in the RAR following renewal. Test system consisted of natural water from a deep well and soil, which is not in accordance with EPA Guideline § 162-3 nor OECD TG 308 as both require the use of sediment. The study summaries note that prior to start of the experiments the microorganisms in the soils were activated for 14 days. In the RAR it is not further elaborated how the activation was achieved, and if it implies that microorganisms were pre exposed to bentazone before tests start. The latter is not allowed, as it could lead to adaptation and increased biodegradation. In both studies, the test concentrations were set at 9.8 mg/kg soil and the test were conducted in Erlenmeyer flask containing 10 grams soil and 90 mL water. Incubation occurred in the dark at 25 ± 1°C under continuous shaking. Sterile controls were included. Duplicate samples were taken after 0, 15, 30, 60, 90, 210, and 374 days in the aerobe test, and after 0, 30, 60, 90, 262 and 365 days in the anaerobe test. Following extraction, radioactivity was determined by LSC and TLC. Mass balance ranged 90.5-99.8% applied radioactivity (% AR) for the aerobe and 92.9-98.8% AR for the aerobe and anaerobe test, respectively. In the aerobe test, two water/soils systems were tested. It was shown that the amount of radioactivity in soil increased over time, but that the bulk remained in the water phase. At test end, up to 14.2% AR could not be extracted from the soils. The main component identified in soils and water was bentazone, with some very minor amounts of more transformation products. The mineralization rate was very low small, amounting to 0.4 and 0.8% AR after 90 days and 3.1 and 4.5% AR within 374 days. It was concluded that microbial degradation and hydrolysis of bentazone evidently play a subordinate role in the aquatic system. During the renewal procedure, the RMS concluded that this study is considered acceptable for information on the route of degradation, but the acceptability for quantitative assessment of the degradation in water sediment could not be verified. In the anaerobe test, a similar pattern was observed. Most radioactivity remained in the water phase and was attributed to bentazone. Bentazone shifted to the soil reaching 9.8% AR at test end (365 days). Practically no mineralization occurred under anaerobic conditions. After 365 days, 87.7 % in the water phase and 5.5 % in the soil were assigned to bentazone. Half-lives could not be determined. The Dossier submitter notes that Gerhardt and Hamm (1987) used soil instead of sediment, and that it is unclear how the microorganisms were activated. Nonetheless, this study does show that degradation hardly occurred in this water/soil system with high levels of bentazone after approximately

one year. The Dossier Submitter considers the results as reliable with restrictions (Klimisch score of 2). **Results are used as supportive for the low biodegradation potential of bentazone.**

Bieber (1994) performed a water/sediment simulation study with ^{14}C-bentazone (purity not reported) under aerobe conditions according to BBA guideline IV, 5-1, a German guideline that served as a basis for OECD TG 308. The study was considered acceptable in the RAR. Two water/sediment systems were tested for 100 days at 20°C in the dark. Test concentration was 0.34 mg /L. Limited details on test setup, i.e. number of replicates, and sampling schedule were not specified. LSC, TLC and/or HPLC were used to analyse water, sediment and CO_2/volatile substances. Sterile control was included that remained sterile till test end. Mass balances ranged 87.7-97.2.8% AR. At test end, mineralization amounted to 2.6% AR in both systems. The amount of test substance was 62-69% AR with the largest part being in the water phase. One transformation product was detected at >10% AR, i.e. N-methylbentazone, reaching maximum of 13% AR in one of the systems with the formation being reversible. Non-extractable residue amounted to approximately 15% AR in both systems at test end. The total system DT50 values for bentazone were reported to be 523 and 908 days. Matejek (2012a) performed a kinetic evaluation of the water/sediment study by Bieber (1994) and reported the following DT50 values that were considered acceptable in the RAR: Degradation $DT50_{, \text{total System}}$: 688 and 940 days; Dissipation $DT50_{, \text{water}}$: 701 and 678 days; Dissipation $DT50_{, \text{sediment}}$: 595 and 568 days, respectively. The Dossier Submitter considers the results as reliable (Klimisch score of 1). **Results are used as supportive for the low biodegradation potential of bentazone.**

De Vries (1996) performed a non-guideline water/sediment simulation study with ^{14}C-bentazone (radiochemical purity 99.5-100%) under aerobe conditions. The study was considered acceptable in the RAR. Two water/sediment systems were tested for 106 days under a light/dark regime. After a pre-incubation period of 8 weeks ^{14}C-bentazone was added to the water/sediment samples at a concentration of 0.48 mg/L. Control was included. Duplicate samples were taken at days 0, 8, 14, 28, 35, 42, 71, and 106. LSC and TLC were used to analyse water, sediment and CO_2/volatile substances. At test end ionspray-MS/MS was used to confirm absence transformation products. Mass balances ranged 90.9-107.7% AR, except for single time point measurement for both systems that were too low, and that were left out. Mineralization was 1.34% AR. Transformation products remained <5.5% AR. Non-extractable residues in sediment <10.5% AR. After 8 days equilibrium was reached for bentazone in water/sediment system, and till test end there was no decline in bentazone concentration. DT50 could not be determined. The Dossier Submitter considers the results as reliable with restrictions (Klimisch score of 2). **Results are used as supportive for the low biodegradation potential of bentazone.**

11.1.4.3.3 Biodegradation in soil

The RAR contains numerous studies that investigated for bentazone the route and rate of degradation in soil under aerobe and/or anaerobe conditions. The respective summaries from the RAR can be found in Annex I. Below an overview is provided of the relevant studies, supplemented with the Dossier Submitter's comments where applicable. The field studies are not discussed below. A general comment is that according to OECD TG 307, studying one soil suffices to evaluate transformation pathways, while three or more additional soils are needed to determine rates of transformation. Most of the studies reported in RAR did not investigate four soils, thus not meeting the OECD TG 307 requirement. Furthermore, all studies reported dissipation DT50 values (sometimes in the RAR referred to as DegT50) where the DT50 was calculated using the concentrations of bentazone that could be extracted from the soils thus not accounting for non-extractable residues (NER). The amount of NER varies depending on the harshness of the extraction method, but in most studies with bentazone NER formation was reported to be substantial at test end. To assess persistence degradation DT50 values are required though that represent mineralization and/or primary degradation, and not disappearance due to adsorption.

During the renewal in 2013 the RMS concluded in the RAR the following with regard to Drescher and Otto, (1972; 1973), Anonymous (1974) and Keller (1987, 1988): "*The studies were considered acceptable in the original DAR however, based on the reduced information available 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*". The Dossier Submitter agrees that for Drescher and Otto (1972; 1973) and Anonymous

(1974) insufficient information is provided to assess their reliability. The results are assigned a Klimisch score of 4, and are not further discussed.

Regarding Keller (1987), the amount of information provided in the summary is similar to that of studies that were considered acceptable or additional in the RAR. Keller (1987) investigated the aerobic metabolism pathway for ^{14}C-bentazone (purity not reported) in three soils, i.e. sandy loam, loamy sand and clay soil. This non-guideline study was conducted in glass reactor consisting of a metal rack (10 trays) with max. 10 petri dishes containing 100 g soil per tray. Test concentration was 10 mg/kg bentazone. Following spiking, soil moisture was adjusted to 40% maximal water holding capacity (MWHC). Samples were taken at days 0, 3, 7, 14, 28, 60, 90, 180, 270 and 360. Volatiles from trapping system were analysed with LSC and soil with TLC. Mass balance was 83-98.9, 89.7-110.7 and 77.2-87.8% AR for the three soils, which is for two soils below 90-110% AR as recommended by OECD TG 307 for studies with radiolabelled test material. No transformation products were detected in the sandy loam, loamy sand and clay soil until day 90, 60 and 60, respectively. After 90 days 43-74% AR could not be extracted from the soils. Mineralization was reported to be 6-9% AR after 90 days and 12-24% AR after 360 days. The Dossier Submitter notes that a non-guideline setup was followed and that for two soils too low total recovery was obtained (<90% AR). These data are considered unreliable (Klimisch score of 3). For the soil with mass balance in the range 89.7-110.7% AR, the results could be used. However, from the RAR summary it cannot be determined which results belong to that soil, and thus these data are considered unassignable (Klimisch score of 4).

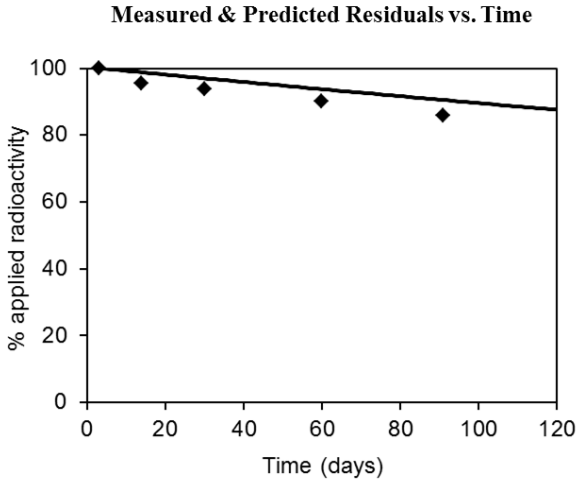
Regarding Keller (1988), degradation behaviour of bentazone (purity not reported) was examined in four soils (loam,2x loamy sand and sandy loam) according to BBA Guideline IV, 4-1. Duplicate samples were taken at day 0, 2, 4, 8, 16, 32, 64 and 100. Samples were analysed by GC and S-FPD. Setup was not further detailed, nor were results presented. This study is also considered unassignable (Klimisch score of 4).

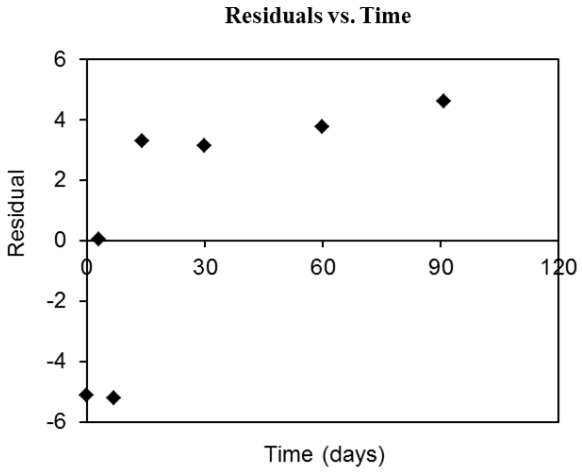
Ebert (1995) studied the degradation of ^{14}C-bentazone (purity 99.6%; radiochemical purity >99 %) in a loamy sand soil using a lysimeter according to SETAC (1995). A soil layer (0-34 cm) was taken and 1.3 mg/kg bentazone was applied. The soil layer was incubated at 20 °C and 40% MWHC. Samples were taken at days 0, 3, 7, 14, 30, 60, 91 and 183. Soil and volatiles (trapping system) were analysed with LSC and HPLC. Mass balance ranged 96.5-105.6% AR. After 91 days, mineralization amounted to 10.6% AR and 73.3% AR was reported to be non-extractable. After 183 days, mineralization amounted to 11.7% AR. Low levels of transformation products were detected, with the highest being 5.8% AR for N-methyl bentazone at test end. A DT50 of 16.9 days was reported. Budde (2014a) performed a kinetic evaluation of Ebert (1995) according to FOCUS (2006) and reported a normalized DegT50 of 15.9 days. This value was considered acceptable in the RAR. The Dossier Submitter notes that a lysimeter study is not a soil simulation study. Furthermore, the DT50/DegT50 calculations by Ebert (1995) and Budde (2014a) are considered dissipation DT50 where adsorption to organic material strongly determines disappearance of bentazone. A degradation DT50 value can be calculated by assuming that the non-extractable residues represent parent substance (see table below). Using FOCUS degradation kinetics (v2), a Single First Order (SFO) fit would yield a degradation DT50 of 610 days. The visual fit appears good, but assessment is hampered as degradation reaches maximally 15% AR after 183 days. The same limitations apply for the assessment of residual scattering. The χ^2 error value is low (3.4%). Furthermore, only one soil was tested. Overall, the degradation DT50 of 610 days at 20°C should be considered indicative for the slow degradation potential of bentazone. Furthermore, extrapolation to an environmentally relevant temperature of 12°C would result in a degradation DT50 of 1295 days. Results are considered reliable with restrictions (Klimisch score of 2), and are **used as supportive for the low biodegradation potential of bentazone.**

Table 11. Aerobic degradation of bentazone

Day	bentazone (% AR)	Non-extractable residues (% AR)	Sum
0	102.6	2.9	105.5
3	83.3	16.7	100
7	74.8	30	104.8
14	56.5	39	95.5

30	34	59.9	93.9
60	16.4	73.6	90
91	12.5	73.4	85.9
183	11.2	75	86.2



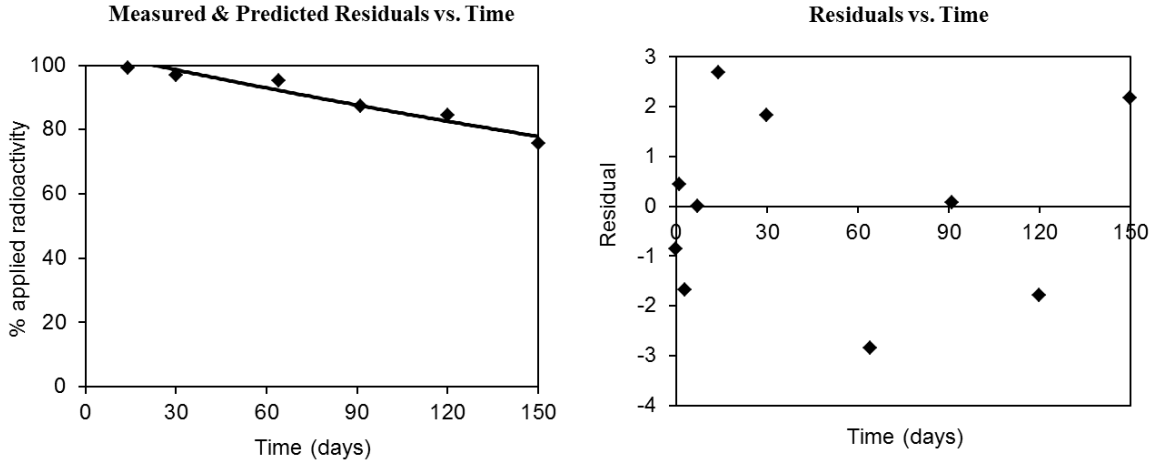


Staudenmaier and Kuhnke (2010b) studied aerobic degradation of ^{14}C-bentazone (radiochemical purity 96.5%) in a sandy loam soil according to OECD TG 307. The study was considered acceptable in the RAR. The test was performed using nominal test concentration of 2.7 mg/kg at 20 °C in the dark for 126 days in a closed incubation system with continuous aeration. Test vessels contained 100 g (d.w.) soil with moisture content of 40% MWHC. Microbial viability of soil was verified at day 57 and 126. Samples were taken at days at 0, 1, 3, 7, 14, 30, 64, 91, 120 and 150. LSC and HPLC were used to analyse soil. Mass balance ranged 92.6 to 105.5% AR, except at test end where it amounted to 84.7% AR. Mineralization was 9.0% AR after 150 days. Besides $^{14}CO_2$, no other volatile compounds were detected. Transformation products were detected in minor amounts, i.e. highest level was 2.8% TAR for N-methyl-bentazone, sum of the other remained below 2.2% AR at all sampling times. Non-extractable residues increased over time, from 3.3% AR at day 0 to 68.8% AR after 150 days. A DT50 of 45.1 days was estimated by SFO kinetics. The Dossier Submitter notes that the reported DT50 represent dissipation. Mineralization (9% AR after 150 days) and amount of transformation products formed (max. 5% AR at day 120) were limited. The disappearance is mostly due to adsorption to organic material. In the RAR it is noted that the part of the non-extractable fraction that could be further investigated consisted mostly of parent substances. Therefore, the approach followed by Staudenmaier and Kuhnke (2010b) overestimates degradation potential of bentazone. By assuming that e non-extractable residues represent parent substance (see table below) a degradation DT50 of 352 days at 20°C can be calculated. The SFO fit (FOCUS degradation kinetics (v2)) appears visually good, but assessment is hampered as degradation reaches maximally 25% AR at test end. The χ^2 error value is low (1.5%), and residuals appear randomly scattered. Extrapolation to 12°C would yield a degradation DT50 of 747 days. Results are considered reliable (Klimisch score of 1), and are **used as supportive for the low biodegradation potential of bentazone.**

Table 12. Aerobic degradation of bentazone

Day	bentazone (% AR)	Non-extractable residues (% AR)	sum
0	102.2	3.3	105.5
1	98.2	5.8	104
3	96.9	8.8	105.7
7	91.8	11.4	103.2

14	83.8	15.3	99.1
30	71.9	24.9	96.8
64	46.1	49	95.1
91	30.5	56.9	87.4
120	20.1	64.3	84.4



Völkel (2001) investigated the aerobic degradation of ^{14}C-bentazone (purity 99.9%; radiochemical purity 99.8%) in a loamy sand soil according to the draft OECD TG 307 (2000). The test was performed using nominal test concentration of 1.46 mg/kg at 20 °C for 117 days with continuous aeration. Flasks with air in- and outlet were filled with 100 g (d.w.) soil with moisture content of 40% MWHC. Traps were included. Samples were taken at days at 0, 1, 3, 7, 14, 30, 56 and 117. Biomass was determined at test start and end. LSC, HPLC and TLC were used to analyse soil and volatiles. Mass balance ranged 92.0 to 97.5% AR. Mineralization reached maximum of 14.9% AR after 117 days. Besides $^{14}CO_2$, no other volatile compounds were detected. Transformation products were not identified, but it was reported that at least eight minor fractions were observed, none of them exceeding individually 2.1% of AR. Non-extractable residues increased over time, from 0.8% AR at day 0 to 53.5% AR after 117 days. For bentazone, a DT50 of 9.6 days was estimated by SFO kinetics. In the RAR the RMS stated that while the study was conducted according to guidelines preceding current guidelines, it is considered acceptable as the measurements and results are in line with current guidelines. The Dossier Submitter notes that the reported DT50 represent dissipation. A degradation DT50 cannot be calculated as it is unclear to what extent the bentazone was present in the system, i.e. a fraction named bentazone is shown, but also a harsh extraction fraction (M7) that consists of at least four, not further identified, radioactive fractions. The results are considered reliable with restrictions (Klimisch score of 2). The dissipation DT50 is not useful for classification purposes, and a degradation DT50 could not be determined. **The amount of mineralization (14.9% AR after 117 days) does show that bentazone is not quickly mineralized, and this can be used as supportive data.**

Ebert (2010b) investigated anaerobic degradation of ^{14}C-bentazone (purity 99.9%; radiochemical purity 99.8%) in a sandy loam soil according to OECD TG 307. This GLP-compliant study was considered acceptable in the RAR. The test was performed using a nominal test concentration of 2.4 mg/kg in soil adjusted to 40% MWHC, and was performed at 20 °C in the dark. Soil were pre-incubated under aerobic conditions for 14 days, after which soils were flooded and aeration was switched to nitrogen to expose the soils till day 120 under anaerobe conditions. Type of test vessels and amount of soil were not specified. Microbial biomass was 38.7 mg C/100 g d.w. soil at start. Samples were taken at days at 0, 3, 7, 14, 21, 28, 42, 77 and 120. LSC and HPLC were used to analyse soil and trapped Co_2/volatiles. Mass balance ranged 100.1 to 102.7% AR. Mineralization reached 6.5% AR at test end. Besides $^{14}CO_2$, no other volatile compounds were detected. No transformation product exceeded 1% AR at any sampling time. Non-extractable residues increased during the 14-day pre-incubation period from 1.9% AR at day 0 to 39.3% TAR after 14 days. During the subsequent anaerobe phase the amount of non-extractable residues remained

stable in the range 39.5-43.2% AR. In the RAR, a DT_{50} of 18.5 days was reported for the aerobic incubation, whereas for the anaerobic phase no degradation was observed (DT_{50} > 1000 days). The Dossier Submitter notes that the reported DT50 represent dissipation. The RAR summary only notes that transformation products were not formed at>1% AR, but measurements are not shown. Considering that mineralization was limited to 5.6% AR after 120 days, and that it primarily took place under aerobe conditions (4.7% AR after 14 days), the **results can be used as supportive for the low biodegradation potential of bentazone under anaerobe conditions.**

Tornisielo and Sacchi (2011b) investigated aerobic degradation of ^{14}C-bentazone (radiochemical purity 99.1%) in two loamy sand and two sandy loam soils according to OECD TG 307. This GLP-compliant study was considered acceptable in the RAR. The test was performed using a nominal test concentration of 2 mg/kg in soil adjusted to 40% MWHC, and was performed at 20 °C in the dark. A closed incubation system with continuous aeration (moistened air) was used with an attached trapping system for volatiles. Type of test vessels and amount of soil were not specified. Microbial biomass was determined after 62 and 120 days, ranging 19 to 41 mg C/100 g d.w. soil, confirming microbial viability of the soils. Samples were taken at day 0, 3, 7, 14, 29, 62, 90, and 120. LSC and HPLC were used to analyse soil and trapped Co_2/volatiles. Mass balance ranged 91.8% to 111.8% AR for all soils. Mineralization ($^{14}CO_2$) reached 9.1 to 21.2% AR after 120 days in the four soils. No other volatile compounds were detected. Formation of non-extractable residues increased in all four soils from 2.2 - 2.9% AR at day 0 to 65.2 - 91.3% AR at test end (day 120). The extractable fraction consisted predominantly of bentazone at all tame points. Transformation products were limitedly formed with N-methyl-bentazone being detected in three soils with maximum concentration reaching 0.5, 2.2 and 5.4% AR at test end, and two minor unknown transformation products, reaching maximum values of 1.5 and 2.2% AR. Dissipation DT50 values for bentazone were calculated using SFO and were reported to be 30.9, 33.0, 43.4 and 49.1 days at 20°C. The Dossier Submitter agreed that the reported DT50 represent dissipation. Four soils have been studied, which is in accordance with OECD TG 307. The results are considered reliable (Klimisch score of 1) and **results can be used as supportive for the low biodegradation potential of bentazone under aerobe conditions.**

Considering the soil degradation data, it can be concluded that bentazone is slowly degraded in soil, and that determination of degradation DT50 values is hampered by adsorption of bentazone to soil.

11.1.4.4 Photochemical degradation

There are four studies in the RAR that investigated aqueous photolysis.

Singh (2011a) investigated the aqueous photolysis of radiolabelled ^{14}C-bentazone (purity not reported; radiochemical purity: 97.3%) in buffered solutions with pH 5, 7 and 9 at a temperature of 22 ± 1°C according to OECD TG 316. Test concentrations were 17.8 mg/L at pH 5 and 7, and 16.7 mg/L at pH 9. Test solutions were continuously exposed to artificial sunlight (light intensity: 618 W/m^2) for 15 days. Dark control samples were included to determine if hydrolysis occurs. Sterile conditions were maintained. Duplicate samples were analysed by LSC and HPLC at day 0, 3 (4), 6, 10 (11), 13, and 15. For the isolation and identification of the ^{14}C degradation products aliquots of the photolysis samples were analysed by LC/MS/MS. Bentazone was shown to rapidly degrade in sterile water under photolytic conditions with degradation half-lives of about 3.3, 5.4 and 3.9 days in pH 5, 7 and 9 buffer solutions, respectively. More than 15 photo-products were formed, two of them exceeding 10% of the total applied radioactivity (TAR). These two peaks were designated as PeakB (max. 30% TAR at pH 5; 3-isopropyl-2,3-dioxo-5-oxocyclopenteno [d]-1 H-2,1,3-thiadiazine-4(3H)-one-6-carbonic acid), and PeakC (max. 25% TAR at pH 7; 1-[N-methylethyl]-1-sulfoamino-benzamide, later identified as sodium 2-[(isopropyl-amino)-carbonyl] phenylsulfamate). The rest of the degradation products were minor and did not exceeded 5% TAR. Bentazone was stable in test buffers (pH 5, 7 and 9) under dark conditions.

Housari et al. (2010a) investigated the aqueous photolysis of bentazone (purity 99.7%) in a non-guideline study using filtered (0. 45µm) natural canal and lagoon (=brackish) water. Test duration was 8 days exposed to natural sunlight under summer conditions in Marseille (France). Test concentration was 50 µM. Control was included consisting of ultra-pure water with test concertation of 10 µM incubated in natural sunlight and dark. Temperature and pH were not reported. Analytical measurements conducted, with recoveries 83 to

90% of nominal concentrations. The overall half-life times for bentazone were 2.17 ± 0.25 and 4.08 ± 0.32 days 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 days. The study estimated a half-life of 12 days in lagoon water under field conditions.

Dawson, Lynn & McCorquodale (2003) investigated the aqueous photolysis of ^{14}C-bentazone (purity: 99%; radiochemical purity: 99.3%) in a buffered solution with pH 7 at a temperature of 20 ± 3°C.Test guideline was not specified. Test concentration in the main study was 5.29 µg/mL. Test solutions were continuously exposed to artificial sunlight (light intensity: 456 W/m^2) for 7 days. Dark control was included. Sterility was not monitored. Duplicate samples were analysed by LSC, TLC and HPLC at 0, 12, 24, 48, 96 and 168 hours. Overall recoveries ranged 92.7-99.04%. At test end 7% of radioactivity was characterised as parent substance, 7 photolytic degradation products were identified, of which 4 exceeding 10% of the total applied radioactivity with the maximum levels amounting to 19% for unknown 1 (= several minor components), 24% for unknown 4 (= 3-isopropyl-2,3-dioxo-5-oxocyclopenteno[d]1 H-2,1,3-thiadiazine-4(3H)-one 6 carbonic acid) , 13% for unknown 5 (= 2-[(isopropylamino)carbonyl]phenylsuIfamic acid) and 13% ofr unknown 6 (=8-hydroxy bentazone). The DT50 value was calculated as 47.8 h; the corresponding DT90 value was 158.8 h.

Philips and Dawson (2002) did not investigate aqueous photolysis, but identified photolytic degradation products detected in the above studies.

Considering the above studies, it can be concluded that aqueous photolysis of bentazone occurs rapidly under laboratory conditions with half-lives in the range of 2.0 to 5.1 days. However, it should be kept in mind that the light conditions for the photo-degradation experiments in glass vials differ from those in natural waters that can be deep and turbid. Furthermore, Singh (2011a) and Dawson, Lynn & McCorquodale (2003) exposed continuously while under realistic conditions light exposure can be limited especially in the winter period. **Overall, the photochemical degradation data show that photolysis of bentazone in water occurs rapidly under laboratory conditions. Under environmentally relevant conditions, e.g. deeper and more turbid waters with lower light intensities, photolysis is expected to be a less relevant degradation route.**

Summary of data /information on rapid degradability

The biodegradation potential of bentazone has not been evaluated using a ready biodegradability test. In a biodegradability screening study where sediment was used as inoculum instead of activated sludge, and where exposure duration was 117 days instead of 28 days primary degradation was not observed (0% at 10 and 20 mg/L). Low biodegradability is supported by QSAR estimations with BIOWIN estimating bentazone as not readily biodegradable. Simulation data for the surface water compartment is not available. Degradation in water/sediment systems is very slow with DT50 values for the sediment of 568-595 days at 20°C, and total system DT50 of 688-940 days at 20°C. Correction to an EU relevant temperature of 12°C would result in DT50 for sediment of 1205-1263 days, and DT50 total system of 1460-1497 days. The DT50 values reported for soil concern dissipation and not degradation, as non-extractable residues (NER) were not accounted for. The available soil degradation data show that bentazone is slowly degraded in soil, and that determination of degradation DT50 values is hampered by adsorption of bentazone to soil. The main component identified in water was bentazone, with one water-sediment study reporting N-methylbentazone, reaching a maximum of 13% AR, and the formation being reversible. Photochemical degradation studies identified three other transformation product that might not be formed under environmentally relevant conditions, i.e. 3-isopropyl-2,3-dioxo-5-oxocyclopenteno[d]1 H-2,1,3-thiadiazine-4(3H)-one 6 carbonic acid, 2-[(isopropylamino)carbonyl]phenylsuIfamic acid) and 8-hydroxy bentazone. Overall based on this information, it can be concluded that bentazone should be considered as not rapidly biodegradable for classification purposes.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this dossier.

11.2.1 Summary of data/information on environmental transformation

Not relevant for this dossier.

11.3 Environmental fate and other relevant information

11.3.1 Adsorption/Desorption

The sorption behaviour of bentazone has been extensively studied in several batch equilibrium studies. In the RAR 12 studies have been assessed and it was concluded that the sorption of bentazone to soil is low. The organic carbon related sorption coefficients of bentazone in soil ($K_{f,oc}$ values) were reported to range from 3.0 to 175.6 L/kg, with a median $K_{f,oc}$ value of 25.2 L/kg. It was further noted that the sorption isotherms showed a considerable non-linearity with a median Freundlich exponent of 0.85. In RIVM report 2015-0095 the leaching of bentazone to ground water was assessed (van der Linden et al., 2015). It was shown that this weak acid shows pH-dependent sorption behaviour in soil. **Overall, b**entazone is mobile in the environment, and is expected to wash-off and leach to the aquatic compartment.**

Table 13. Adsorption values of bentazone in different soils as reported in the RAR

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**		

11.3.2 Votalisation

The RAR reports two Henry's law constant values, i.e. 2.108 x 10^{-6} and 7.2 x 10^{-5} Pa m³/mol. While one value is expected, from both values can be concluded that bentazone will not significantly volatilize from moist soil surfaces to air. Once in the air, bentazone is expected to rapidly react with hydroxyl radicals with an estimated atmospheric half-life of 2.1 hours (AOPWIN v1.91).

11.4 Bioaccumulation

Table 14: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
shake flask methodology (EEC method A8)	log D_{ow}: 1.54 at pH 4 -0.94 at pH 7 -1.32 at pH 9	Highest log D_{ow} well below the threshold of log K_{ow} ≥4 **Supportive data**	Daum 2000b,e[a]
shake flask methodology (EEC method A8)	log D_{ow}: 0.77 at pH 5 -0.46 at pH 7 -0.55 at pH 9	Highest log D_{ow} well below the threshold of log K_{ow} ≥4 **Supportive data**	Keller, 1986[a]
Ready biodegradability Method: BCFBAF (v3.01)	BCF: 16.25 L/kg wwt (regression-based method) BCF: 22.55 L/kg wwt (Arnot-Gobas (upper trophic) method)	QSAR Based on a log K_{ow} of 2.34, which is indicated in KOWWIN as experimentally determined. This is a worst-case approach considering the highest log D_{ow} amounts to 1.54 (which would yield BCF values of 4.82 and 4.39 L/kg wwt, respectively). Klimisch score of 2 **Supportive data**	EPA, 2012
Fish bioconcentration *Lepomis macrochirus* Flow-through (US EPA 165-4 (1982), OECD 305 E (1981))	BCF whole fish: 1.4 L/ kg BCF edible: 0.4 L/ kg BCF non- edible: 2.2 L/ kg	BCF based on total radioactivity. Not corrected for growth, nor normalized to 5% lipid content. **Key data**	Anonymous, 1992a STUDY IIA 8.2.6.1/01

[a] As summarised in the Final addendum to the Renewal Assessment Report for Bentazone, January 2015.

11.4.1 Estimated bioaccumulation

The Dossier Submitter estimated using BCFBAF (v3.01) BCF values of 16.25 and 22.55 L/kg wwt using the regression-based and the Arnot-Gobas (upper trophic) methods, respectively. These values are worst-case estimates considering that a log K_{ow} of 2.34, which is indicated in KOWWIN as experimentally determined, was used as input. Using the highest log D_{ow} of 1.54 reported in the RAR as input, yields BCF values of 4.82 and 4.39 L/kg wwt, respectively. The respective log D_{ow} was determined at a pH where bentazone is primarily neutrally charged, and thus it approaches the log K_{ow}. The applicability domains of the models are not explicitly defined. Bentazone is considered to fall within the applicability domain though, as the

partitioning coefficient (log D_{ow} ranges -1.32 to 1.54) and molecular weight (240.3 g/mol) are within the range of (ionic) training set compounds, i.e. MW of 69 to 992 g/mol, and log K_{ow} of -6.5 to 11.26; and five fragments were identified to estimate the BCF. It should be noted that not all structures were identified, and used for the BCF estimations (i.e. functional groups containing nitrogen and sulphur). Overall, the results are considered as reliable with restrictions (Klimisch score of 2), and can be used as supportive data.

Overall, the estimated BCF values indicate a low bioaccumulation potential.

11.4.2 Measured partition coefficient and bioaccumulation test data

Bioaccumulation of ^{14}C-labelled bentazone (purity >99%; radio purity 97%) was studied in *Lepomis macrochirus* under flow-through conditions according to US EPA 165-4 (1982) and OECD 305 (1981) (Anonymous, 1992a). Bluegill sunfish (mean weight 0.95 g; length 34.2 mm) were exposed to 5 mg/L bentazone for 28 days to measure uptake of the compound and then placed in clean water for 17 days to determine elimination rate. Control and treatment consisted of a single test chamber containing 94 fish. Test was performed at 22 ºC using well-water (pH 6.8 -7, 65 mg $CaCO_3$/L and conductivity 203 µmhos/cm). Renewal rate was 4 volumes/day. Four randomly selected fish were sampled from the treatment chamber on days 1, 3, 7, 10, 14, 21, 28, 29, 31, 35, 38 and 42, and from the control chamber on days 0, 21 and 28, respectively. Water samples were daily taken during uptake, and on day 1 of depuration. Additional fish and water samples were taken for determination of metabolites. Radioactivity was analysed by LSC and radio-TLC. No mortality in control during the exposure phase and one fish died in the elimination phase. Fish showed 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/L. 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. It was reported that the steady state and kinetic BCF values did not differ (but these results were not shown). The RMS noted that the lipid content was not measured, and that the fish length and weight at the end of the test were not reported. Thus, the reported BCF values are not corrected for growth dilution and normalized to 5% lipid content. The Dossier Submitter notes that while metabolites were measured, no data are presented and the reported BCF values are based on total radioactivity. This approach would yield worst-case BCF values if transformation occurred, but that cannot be determined. The BCF values are well below the threshold of 500 L/kg. **Therefore, the experimentally determined BCF values indicate a low potential for bioaccumulation.**

The RAR reports two studies that determined the *n*-octanol water partitioning coefficient of bentazone at acid, neutral and alkaline conditions. Both studies were conducted according to EEC method A8, i.e. shake flask methodology. For non-charged substances the ratio of the concentration in *n*-octanol and water is referred to as the log K_{ow}. The charge of ionisable organic compounds depends on pH, and thus the distribution at a certain pH is referred to as log D_{ow}. The log D_{ow} determined at a pH where the substance is present in the neutral from corresponds to the log K_{ow}. For bentazone log D_{ow} values of 1.54, -0.94 and -1.32 have been determined at pH 4, 7, and 9 (Daum 200b,e) and 0.77, -0.46 and -0.55 at pH 5, 7 and 9 (Keller, 1986), respectively. The pK_a of bentazone has been reported to 2.50 (Hogg, 2001b) and 3.51(Daum, 2000c), and the molecule is increasingly present in the neutral form at pH values below the pK_a. The highest log D_{ow} of 1.54 determined at pH 4, which can be considered representing worst-case for environmental conditions, approaches the log K_{ow}. As the highest log D_{ow} of 1.54 is well below the threshold of log $K_{ow} \geq 4$, **bentazone is considered to have a low potential for bioaccumulation**.

11.5 Acute aquatic hazard

As concluded in section 11.1, bentazone can degrade to *N*-methylbentazone (max 13%) in water-sediment systems, with the formation being reversible. Three other transformation products (>10%) have been identified following aqueous photolysis of bentazone , i.e. 3-isopropyl-2,3-dioxo-5-oxocyclopenteno[d]1 H-2,1,3-thiadiazine-4(3H)-one 6 carbonic acid, 2-[(isopropylamino)carbonyl]phenylsuIfamic acid) and 8-hydroxy bentazone. This occurred under laboratory conditions and it seems unlikely that under environmentally more relevant conditions, e.g. deeper and more turbid waters with lower light intensities, the latter three latter transformation products will be formed in significant amounts. That said, the EFSA conclusion document on bentazone notes a low risk to aquatic organisms for these three transformation products. Regarding, *N*- methylbentazone the EFSA conclusion document notes that is more toxic to fish and aquatic invertebrates than bentazone. In the RAR the following aquatic toxicity data are reported for *N*-methylbentazone: fish 96h-LC50 of 8.56 mg/L (mean measured); fish 28d-NOEC of 0.23 mg/L (nominal with actual ~100% of nominal); aquatic invertebrates 48h-LC50 of 26.5 mg/L (mean measured); daphnia 21d-NOEC of 2.0 mg/L (nominal with actual within 20% of nominal); algae 72h-E_rC50 of 37.7 mg/L (nominal with actual within 20% of nominal); and lemna 7d-E_rC50 of 35.8 mg/L (mean measured). Considering that degradation of bentazone in water is a slow process, that the formation of *N*-methylbentazone is reversible, and that the presence of *N*-methylbentazone will result in a more conservative assessment of bentazone toxicity, the classification will be conducted based on studies conducted with bentazone.

Table 15: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks[1]	Reference
Fish					
Acute toxicity to fish static OECD TG 203	Rainbow trout (*Oncorhynchus mykiss*), old name (*Salmo gairdneri*)	Bentazone (BAS 351 H) Purity: 97.8%	96h-LC_{50}: >97.8 mg/L (corrected for purity)	mortality in limit test, full study required Klimisch score of 3	Anonymous, 1987a
Acute toxicity to fish static OECD TG 203	Bluegill (*Lepomis macrochirus*)	Bentazone (BAS 351 H) Purity: 94%	96h-LC_{50}: >94 mg/L (corrected for purity)	nominal (actual conc.~100 % of nominal) Klimisch score of 1 **Supportive data**	Anonymous, 1986a
Acute toxicity to fish static OECD TG 203	Common carp (*Cyprinus carpio*)	Bentazone-Na (BAS 351 H) Purity: 100%	96h-LC_{50}: >916 mg/L (corrected for sodium)	nominal (actual conc. ~100 % of nominal) Klimisch score of 2 **Supportive data**	Anonymous, 1983

Acute toxicity to fish static OECD TG 203	Fathead minnow (*Pimephales promelas*)	Bentazone-Na (BAS 351 H-Na) Purity: 91.9%	96h-LC_{50}: >104 mg/L (corrected for sodium)	mean measured Klimisch score of 2 **Supportive data**	Anonymous, 2011a
Acute toxicity to fish flow-through ASTM E 729-88; EPA FIFRA-E 540/9-82-024	sheepshead minnow (*Cyprinodon variegatus*)	Bentazone (BAS 351 H-tech a.i.) Purity: 53.0%	96h-LC_{50}: >136 mg/L	mean measured Klimisch score of 2 **Supportive data**	Anonymous, 1991
Aquatic invertebrates					
Acute toxicity to aquatic invertebrates static OECD TG 202	water flea *Daphnia magna*	Bentazone Purity: 94%	48h-EC_{50}: 125 mg/L	nominal Klimisch score of 3	Bias, 1986a
Acute toxicity to aquatic invertebrates static OECD TG 202	water flea *Daphnia magna*	Bentazone (BAS 351 H) Purity: 98.4%	48h-EC_{50}: >98.4 mg/L (corrected for purity)	nominal (actual conc. ~100 % of nominal) Klimisch score of 2 **Supportive data**	Jatzek, 2003b
Acute toxicity to aquatic invertebrates flow-through EPA-E 540/9-82-024, ASTM E 729-88	mysid shrimp *Mysidopsis bahia*	Bentazone (BAS 351 H-Tech a.i.) Purity: 53.0%	96h-LC_{50}: >132.5 mg/L	mean measured Klimisch score of 1 **Supportive data**	Graves and Smith, 1991a
Acute toxicity to aquatic invertebrates flow-through EPA-E 540/9-82-024, ASTM E 729-88	oyster embryo *Crassostrea virginica*	Bentazone (BAS 351 H) Purity: 53.0%	96h-LC_{50}: >109 mg/L	mean measured Klimisch score of 2 **Supportive data**	Graves and Smith, 1992a
Algae or other aquatic plants					
Algal growth inhibition static OECD TG 201	green alga *Ankistrodesmus bibraianus* (current name *Selenastrum bibraianum* Reinsch)	Bentazone (BAS 351 H) Purity: 82.6%	72h-E_bC_{50}: 62 mg/L	nominal Klimisch score of 3	Dohmen, 1990a

Algal growth inhibition static OECD TG 201	green alga *Ankistrodesmus bibraianus* (current name *Selenastrum bibraianum* Reinsch)	Bentazone (BAS 351 H) Purity: 53.0%	72h-E_bC_{50}: 71 mg/L (corrected for purity)	nominal Klimisch score of 3	Dohmen, 1990b
Algal growth inhibition static OECD TG 201	*Pseudokirchneriella subcapitata* (current name *Raphidocelis subcapitata*)	Bentazone (BAS 351 H) Purity: 98.4%	72h-E_rC_{50}: 32.8 mg/L 72h-E_rC_{10}: 9.7 mg/L (corrected for purity)	nominal (actual conc. ~100% of nominal, but determined in replicate without algae) Klimisch score of 2 **Supportive data**	Jatzek, 2003b
Algal growth inhibition static ISO guideline 8692:2012	*Pseudokirchneriella subcapitata*	Bentazone Purity: >90%	48h-E_rC_{50}: 13.6±2.3 mg/L	nominal Klimisch score of 4	Cedergreen and Streibig, 2005
Algal growth inhibition static ISO guideline 8692:2012	*Pseudokirchneriella subcapitata*	Bentazone Purity: not reported	48h-E_rC5_0: 0.051 ± 0.004 mg/L	nominal Klimisch score of 4	Munkegaard et al., 2008
Algal growth inhibition static ISO guideline 8692:2012	*Skeletonema costatum*	Bentazone Purity: not reported	72h-E_rC50: 24.0 mg/L	nominal Klimisch score of 4	Macedo et al. (2008)
Algal growth inhibition static non-guideline	*Chaetoceros gracilis*	Bentazone Purity: not reported	72h-E_rC50: 150 mg/L	nominal Klimisch score of 4	Hourmant et al. (2009)
Lemna growth inhibition static non-guideline	*Lemna gibba*	Bentazone (BAS 351 H) Purity: 53.0%	14d-E_yC_{50}: 5.35 mg/L	mean measured Klimisch score of 2 **Supportive data**	Hughes, 1991a
Lemna growth inhibition static OECD TG 221	*Lemna gibba*	Bentazone Purity: 100%	**7d-E_rC50: 12.0 mg/L (dry weight)**	mean measured Klimisch score of 1 **Key data**	Hoffmann, 2011b

Lemna growth inhibition static OECD TG 221 OPPTS 850.4400 (draft)	*Lemna gibba*	Bentazone-Na Purity: 91.9%	7d-E_rC50: 17.0 mg/L (dry weight)	mean measured Klimisch score of 1 **Supportive data**	Hoffmann, 2011a
Lemna growth inhibition static ISO guideline 20079:2005	*Lemna minor*	Bentazone Purity: >90%	7d-E_rC10: 1.14±2.20 mg/L (frond area)	nominal Klimisch score of 4	Cedergreen and Streibig (2005)
Lemna growth inhibition static ISO guideline 20079:2005	*Lemna minor*	Bentazone Purity: not reported	7d-E_rC10: 1.14±2.20 mg/L (frond area)	nominal Klimisch score of 4	Munkegaard et al. (2008)

[1] Studies indicated as key data refer to the most conservative study; other reliable experimental information is referred to as supportive data.

11.5.1 Acute (short-term) toxicity to fish

There are five studies available in the RAR that investigated the acute toxicity of bentazone to fish.

Anonymous (1987a) performed a GLP-compliant 96-hours static test with bentazone (purity of 97.8%) using Rainbow trout (*Oncorhynchus mykiss*) according to OECD TG 203 (Document IIA/ Section 8.2.1.1/01). Two nominal concentrations were tested: the 100 mg/L treatment in triplicate, and the 50 mg/L treatment (as well as the control) without replicates. Test vessels were 100-L glass aquaria containing 10 fish (mean body length of 7.4 cm; mean wet weight of 2.6 g). Medium was reconstituted freshwater according to DIN 38 412, Part 11, with hardness of 2.5 mmol/L, dissolved oxygen levels of 9.8-11.0 mg/L and pH 7.0-8.0. Loading rate corresponded to 0.26 g fish/L. Continuous aeration was applied. Fish were not fed during testing. Temperature was maintained at 12°C. Observations were performed after 1, 4, 24, 48, 72 and 96 hours. Analytical monitoring was performed after 1.5 and 96 hours using HPLC. Actual bentazone concentrations ranged 100.4-101.2% of nominal after 1.5 hours, and 100.2-101.4% at test termination. Actual concentrations were above 80% of nominal, and the results were expressed as nominal concentrations in the RAR. There was no mortality in the control and the 50 mg/L treatment, while 10% mortality was reported for the 100 mg/L treatment. Sublethal effects were not observed in the control nor the treatments. The 96h-LC50 of bentazone was expressed as >100 mg/L. Study was considered acceptable in the RAR. The dossier submitter notes that if any mortality is observed in a limit test, a fully study should be conducted (see section 20, OECD TG 203). The data from this limit test should thus not be used for LC50 derivation. The results are not used for classification purposes.

Anonymous (1986a) performed a GLP-compliant 96-hours static test with bentazone (purity of 94%) using Bluegill (*Lepomis macrochirus*) according to OECD TG 203 (Document IIA/ Section 8.2.1.2/01). Two nominal concentrations were tested: the 100 mg/L treatment in triplicate, and the 50 mg/L treatment (as well as the control) without replicates. Test vessels were 50-L glass aquaria containing 10 fish (mean body length of 4.2 cm; mean wet weight of 0.9 g). Medium was reconstituted freshwater according to DIN 38 412, Part 11, with hardness of 2.5 mmol/L, dissolved oxygen levels of 9.8-11.0 mg/L and pH 7.0-8.0. Loading rate corresponded to ~0.2 g fish/L. Continuous aeration was applied. Not specified if fish were fed. Temperature was maintained at 23°C. Observations were performed after 1, 4, 24, 48, 72 and 96 hours. Analytical monitoring was performed after 1 and 96 hours using HPLC. Actual bentazone concentrations ranged 97.2-100.3% of nominal after 1 hour, and 100.2-101.1% at test termination. As actual concentrations were above 80% of nominal, results were expressed as nominal concentrations. Neither lethal nor sublethal effects were

observed in the control or any of the treatments. The 96h-LC50 of bentazone was expressed as >100 mg/L. Study was considered acceptable in the RAR. The dossier submitter notes that it is not possible to calculate mean measured test concentrations based on the information provided in the RAR summary. This is not considered an issue, since the measured test concentrations were around 100% of nominal. However, the nominal test concentration should be corrected for purity resulting in 96h-LC50 of >94 mg/L. The results can be used for classification purposes.

Anonymous (1983) performed a GLP-compliant 96-hours static test with bentazone-Na (purity of 100%) using common carp (*Cyprinus carpio*) according to OECD TG 203 (Document IIA/ Section 8.2.1.2/02). Four concentrations were tested with the nominal test concentrations being 180, 320, 560 and 1000 mg bentazone-Na/L. Control was included. All treatments, including control, were tested without replicates. Test vessels were 50-L glass aquaria containing 10 fish (mean body length: 5.5 cm (4.6 - 6.3 cm); mean body weight: 2.5 g (1.3 - 3.5 g)). Medium was reconstituted freshwater according to DIN 38 412, Part 11, with hardness of 2.5 mmol/L, dissolved oxygen levels of 7.7-8.3 mg/L and pH 7.7-8.2. Loading rate corresponded to 0.5 g fish/L. Continuous aeration was applied. Not specified if fish were fed. Temperature was maintained at 25°C. Observations were performed after 1, 4, 24, 48, 72 and 96 hours. Analytical monitoring was performed after 1 and 96 hours using UV-spectral photometry. Actual bentazone-Na concentrations ranged 101-102%, 102-103%, 102-104%, 101-102%, and 100-101% of nominal test concentrations after 1, 24, 48, 72 and 96 h, respectively. As actual concentrations corresponded to nominal, results were expressed as nominal concentrations. Neither lethal nor sublethal effects were observed in the control or any of the treatments. The 96h-LC50 of bentazone-Na was expressed as >1000 mg/L. Study was considered acceptable in the RAR. The dossier submitter notes that replicates were not included, but as more than seven fish were tested per treatment OECD TG 203 requirements were met. It was not possible to calculate mean measured test concentrations based on the information provided in the RAR summary. This is not considered an issue, since the measured test concentrations were around 100% of nominal. Purity was 100%, so only a correction for sodium is deemed necessary, resulting in a 96h-LC50 for bentazone of >916 mg/L. The results can be used for classification purposes.

Anonymous (2011a) performed a GLP-compliant 96-hours static limit test with bentazone-Na (purity of 91.9%) using fathead minnow (*Pimephales promelas*) according to OECD TG 203 (Document IIA/ Section 8.2.1.2/03). One nominal test concentration of 120 mg/L (110 mg bentazone-Na/L after correction for purity) was tested in triplicate. Control was conducted in duplicate. Each 10-L test vessel consisted of 10 fish (body length control fish: 1.5-2.2 cm; mean wet weight control fish: 0.06-0.17 g). Medium was non-chlorinated charcoal filtered tap water mixed with deionised water, total hardness 100 mg $CaCO_3$/L and pH 7.5-8.5. Dissolved oxygen levels were not reported. Loading rate corresponded to 0.12 g fish/L. Aeration was not applied. Fish were not fed during testing. Temperature was maintained at 23°C. Observations were performed after 1, 6, 24, 48, 72 and 96 hours. Analytical monitoring was performed at start, 48 and 96 hours using UV-HPLC. Actual bentazone-Na concentrations ranged 99.8-102.3%, 101.9-102.3%, and 105.1-105.7% after 0, 48 and 96 hours, respectively. Neither lethal nor sublethal effects were observed in the control or the treatment. The 96-h LC50 of bentazone was expressed as >113.5 mg/L based on mean measured test concentrations. Study was considered acceptable in the RAR. The dossier submitter notes that dissolved oxygen levels were not reported in the RAR, while this is a relevant water quality parameter. This is not considered a major issue, considering that the RMS concluded in the RAR that water quality parameters were within accepted limits. Correction for sodium yields a 96h-LC50 for bentazone of >104 mg/L. The results can be used for classification purposes.

Anonymous (1991) performed a 96-hours limit test under flow-through conditions with bentazone (purity of 53.0%) using sheepshead minnow (*Cyprinodon variegatus*) according to ASTM E 729-88 and EPA FIFRA-E 540/9-82-024 (Document IIA/ Section 8.11.1/01). One nominal test concentration of 120 mg/L (after correction for purity) and a salt water control were tested in triplicate. Each 6.5-L test vessel consisted of 10 fish (body length control fish: 2.3-3.0 cm; mean wet weight control fish: 0.31-0.96 g). Medium was natural sea water passed through a sand filter and aerated, salinity 25 ‰. Loading rate corresponded to 0.9 g fish/L. Aeration was not applied. Fish were not fed during testing. Temperature ranged 21.8-22.2°C, and pH 8.2-8.4. Dissolved oxygen levels were not reported. Renewal rate was 7.8 volumes/24 hours. Observations were performed after 4, 24, 48, 72 and 96 hours. Analytical monitoring was performed at start, 24, 48, 72 and 96 hours using GC after sample acidification to pH 2 and extraction with methylene chloride. Actual bentazone

concentrations ranged 118.3-127.9% and 137.5-146.2.9% of nominal at test start and end, respectively. Neither lethal nor sublethal effects were observed in the control or the treatment. The 96h-LC50 of bentazone was expressed as >136 mg/L based on mean measured test concentrations. Study was considered acceptable in the RAR. The dossier submitter notes that dissolved oxygen levels were not reported, while this is a relevant water quality parameter. This is not considered a major issue, considering that the RMS concluded in the RAR that water quality parameters were within accepted limits. Dossier submitter further notes that the purity was 53%, and that no information as provided in the RAR with respect to impurities. Since no detrimental effects were observed, the results can be considered reliable with restrictions. The results can be used for classification purposes.

From the above summaries it can be concluded that there are four reliable 96h-LC50 values available for four different fish species, ranging >94 to >916 mg/L. Since all four values are greater than values, no conclusions can be drawn with respect to the most sensitive fish species, and consequently which study should be considered as key study. In this specific case, that is not considered an issue, as aquatic plants appear to be a more sensitive taxon than fish.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

There are four studies available in the RAR that investigated the acute toxicity of bentazone to aquatic invertebrates.

Bias (1986a) performed a 48-hours static test with bentazone (purity of 94.0%) using the water flea *Daphnia magna* according to OECD TG 202 (Document IIA/ Section 8.3.1.1/05). Four concentrations were tested with the nominal test concentrations being 62.5, 125, 250, and 500 mg/L. Control was included. Each treatment consisted of 4 replicates. Test vessels were 250-mL glass beakers containing 5 daphnids (age 6-24 h). Temperature was not reported. Medium was filtered tap water with total hardness of 2.70 ± 0.50 mmol/L; oxygen content: 8.60 mg/L - 9.52 mg/L and pH 3.32 - 8.19. There was no aeration or feeding. Immobility was assessed after 0, 3, 6, 24 and 48 hours. Analytical monitoring was performed using an HPLC method. Sampling regime, not outcome of the measurements were reported. After 48 hours, daphnids were not affected in the control and the lowest treatment, in the 125 mg/L treatment 50% of the daphnids were immobile, and in the 250 and 500 mg/L treatments all daphnids were immobile. An 48h-EC50 of 125 mg/L based on nominal test concentrations is reported. The applicant notes that immobility could at least partly be due to very low pH values at the highest test concentrations (pH < 4). In the RAR this study is considered unacceptable. pH is not a validity criterion, but should be in the range 6 to 9 as specified in OECD TG 202. Thus, the dossier submitter considers the results as unreliable (Klimisch score of 3). The results can not be used for classification purposes.

Jatzek (2003b) performed a 48-hours static test with bentazone (purity of 98.4%) using the water flea *Daphnia magna* according to OECD TG 202 (Document IIA/ Section 8.3.1.1/01). Four concentrations were tested with the nominal test concentrations being 12.5, 25, 50, 100 mg/L. Positive and negative controls were included. Each treatment consisted of seven replicates: four replicates to assess immobility; one replicate to measure pH and oxygen levels at the start; and two replicates to measure actual test concentrations at 0 and 48 hours using HPLC-UV. Test vessels were 250-mL glass beakers containing 5 daphnids (age 6-24 h). Temperature was ranged 18 to 22 °C. Medium was reconstituted M4 synthetic fresh water, total hardness 2.44 mmol/L and pH 8.1. Dissolved oxygen levels were not reported, but RMS reports that water quality criteria were within accepted range. 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. Study was considered acceptable in the RAR. The dossier submitter notes that it is not possible to calculate mean measured test concentrations based on the information provided in the RAR summary. This is not considered an issue, since the measured test concentrations were around 100% of nominal. The nominal test concentration should be corrected for purity, i.e. 48h-EC50 of >98.4 mg/L. The results can be used for classification purposes.

Graves and Smith (1991a) performed a 96-hours limit test under flow-through conditions with technical bentazone (purity of 53.0%) using the mysid *Mysidopsis bahia* according to EPA-E 540/9-82-024, and ASTM E 729-88 guidelines (Document IIA/ Section IIA 8.11.1/02). One nominal test concentration of 120 mg/L (after correction for purity) and a salt water control were tested in triplicate. Each 6.5-L test vessel consisted of 10 mysids. Medium was natural sea water passed through a sand filter and aerated, salinity 25 ‰. Mysids were fed with brine shrimp during testing. Aeration was not applied. Renewal rate was 7.8 volumes/24 hours. Observations were performed after 4, 24, 48, 72 and 96 hours. Temperature ranged 24.7-25.0°C, and pH 8.2-8.3. Dissolved oxygen levels were not reported, but RMS reports that water quality criteria were within accepted range. Analytical monitoring was performed at start, 24, 48, 72 and 96 hours using GC after sample acidification to pH 2 and extraction with methylene chloride. Actual bentazone concentrations ranged 81-169% and 92-131% of nominal at test start and end, respectively. One mortality was observed in both the control and the treatment after 72 hours of exposure. There were no sublethal effects (signs of toxicity or abnormal behaviour).The 96h- LC50 was reported as >132.5 mg/L based on mean measured concentrations. In the RAR, the RMS concluded that actual concentrations are 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. Nevertheless, as actual concentrations were determined, the study was considered acceptable. The results can be used for classification purposes.

Graves and Smith (1992a) performed a 96-hours test under flow-through conditions with bentazone (purity of 53.0%) using the eastern oyster, *Crassostrea virginica* according to EPA-E 540/9-82-024, and ASTM E 729-88 guidelines (Document IIA/ Section IIA 8.11.1/03). Four concentrations were tested with the nominal test concentrations being 15.6, 25.9, 43.2, 72.0, and 120 mg/ L (corrected for purity of the test compound) and a control (salt water) without replicates. Each test vessel contained 20 oysters (mean length 4.7 cm (3.5-4.0 cm); 1-7 mm of the shell was ground off prior to testing) in 12.6 L water. Medium was natural sea water passed through a sand filter and aerated, salinity 25 ‰. Oysters were fed an algal suspension during testing. Aeration was not applied. Renewal rate was 1 L/oyster/h. Observations were performed twice daily. Temperature ranged 24.7-26.1°C, and pH 7.9-8.1. Dissolved oxygen levels were not reported, but RMS reports that water quality criteria were within accepted range. Analytical monitoring was performed daily using GC after sample acidification to pH 2 and extraction with methylene chloride. Actual bentazone concentrations ranged 64-91% of nominal. The mean measured test concentrations were 10, 19, 29, 61 and 109 mg bentazone/L, respectively. 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, respectively. 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-EC50 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. Study was considered acceptable in the RAR. The dossier submitter notes the mean oyster length of 4.7 cm exceeds the reported range of 3.5 to 4.0 cm. Obviously, this is not possible, and has to be an error. Study design was without replicates making it more difficult to obtain reliable effect concentrations. The 96h-EC50 can be used for classification purposes.

From the above summaries it can be concluded that there are three reliable effect concentrations for three aquatic invertebrate species, i.e. a 48h-EC50 of >98.4 mg/L for *Daphnia magna*, a 96h-LC50 of >132.5 mg/L for *Mysidopsis bahia* and a 96h-EC50 of > 109 mg/L for *Crassostrea virginica*. Since all three values are greater than values, no conclusions can be drawn with respect to the most sensitive aquatic invertebrate species, and consequently which study should be considered as key study. In this specific case, that is not considered an issue, as aquatic plants appear to be a more sensitive taxon than aquatic invertebrates.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

There are six studies with aquatic primary producers available in the RAR of which three investigated growth inhibition of bentazone to algae and three to aquatic plants.

Dohmen (1990a) performed a 72-hours static algal growth inhibition test with bentazone (purity of 98.4%) using the green alga *Ankistrodesmus bibraianus* (current name *Selenastrum bibraianum* Reinsch) according to OECD TG 201 (Document IIA/ Section 8.4/03). Eight concentrations were tested with the nominal test concentrations being 0.5, 2.5, 5.0, 10.0, 25.0, 50.0, 75.0 and 100 mg/L. Control was included. Each treatment

consisted of 5 replicates. Test vessels were 100-mL glass Erlenmeyer flasks containing 50 mL OECD medium. Initial cell density was 4 x 10^4 cells/mL. Flask were incubated under continuous light (~8000 lux) and shaking (125 rpm) .Temperature was 21±1°C, and pH ranged 7.75-8.04. Growth was daily assessed. There was no analytical monitoring. A 72h-E_bC50 of 62.0 mg/L was reported based on nominal test concentrations, and based on biomass yield. In the RAR this study is considered unacceptable, because the test item concentrations were not analytically verified, and because test guideline followed was outdated. The results are considered unreliable (Klimisch score of 3). The results can not be used for classification purposes.

Dohmen (1990b) performed a 72-hours static algal growth inhibition test with Bentazone-DEA-salt (purity of 53.0%) using the green alga *Ankistrodesmus bibraianus* (current name *Selenastrum bibraianum* Reinsch) according to OECD TG 201 (Document IIA/ Section 8.4/04). Nine concentrations were tested with the nominal test concentrations being 1.0, 2.5, 5.0, 10.0, 25.0, 35.0, 50.0, 75.0 and 100mg//L (corrected for purity of the test compound). Control was included. Each treatment consisted of 5 replicates. Test vessels were 100-mL glass Erlenmeyer flasks containing 50 mL OECD medium. Initial cell density was 4 x 10^4 cells/mL. Flask were incubated under continuous light (~8000 lux) and shaking (125 rpm) .Temperature was 21±1°C, and pH ranged 7.54-7.9. Growth was daily assessed. There was no analytical monitoring. A 72h-E_bC50 of 71.0 mg/L was reported based on nominal test concentrations (of benthazone), and based on biomass yield. In the RAR this study is considered unacceptable, because the test item concentrations were not analytically verified, and because test guideline followed was outdated. The results are considered unreliable (Klimisch score of 3). The results can not be used for classification purposes.

Jatzek (2003b) performed a 72-hours static algal growth inhibition test with bentazone (purity of 98.4%) using the green alga *Pseudokirchneriella subcapitata* (current name *Raphidocelis subcapitata*) according to OECD TG 201 (Document IIA/ Section 8.4/01). Five concentrations were tested with the nominal test concentrations being 6.25, 12.5, 25, 50, and 100 mg/L in triplicate. Control was tested in five replicates. An additional vessel without algae was included for analytical measurements. Test vessels contained 100 mL medium. Medium was not further specified. Initial cell density was 1x 10^{-4} cells/mL. Vessels were incubated under continuous light 60 – 120 µE/(m^2.s). Temperature was 23°C. Hardness and pH were not reported. Cells were counted after 0, 24, 48, and 72 h by in-vivo chlorophyll fluorescence at 435 nm and counted under a microscope, to determine growth rate and biomass. Analytical monitoring was performed at test start and end using HPLC-UV. Actual bentazone concentrations ranged 101.2-103.8% and 102.4-104.0% of nominal at test start and end, respectively. Growth was exponential in the control (109 fold increase after 72 h). 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. An E_rC50 of 33.3 and an E_bC50 of 16.8 mg/L were reported based on nominal test concentrations, and an E_rC10 of 9.89 mg/L and the E_bC10 of 7.90 mg/L also based on nominal test concentrations. The RMS noted in the RAR that water quality parameters were within acceptable values, and that the control met the validity criteria of the current guideline version. The Dossier submitter notes that the RAR summary only reports on one validity criterion, i.e. exponential growth in the control exceeding a factor 16 within the 72-hour test period. However, no information is provided with respect to the other two validity criteria, i.e. the mean coefficient of variation for section-by-section specific growth rates in the control cultures which should be below 35%, and the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures that should not exceed 7%. In the RAR, the RMS further notes that only a brief description of the study was available and that no validation was reported for the analytical method and no description of the statistical evaluation. The RMS was also not able to determine if the cells were kept in suspension by constant shaking. That said, the study was considered acceptable in the RAR. The dossier submitter notes that it is not possible to calculate mean measured test concentrations based on the information provided in the RAR summary. This is not considered an issue, since the measured test concentrations were around 100% of nominal. The nominal test concentration should be corrected for purity, resulting in an E_rC50 of 32.8 mg/L and an E_rC10 of 9.7 mg/L. It should be noted that the actual concentrations were determined in a replicate vessel without algae. Ordinarily this would invalidate the study as potential dissipation of the substance due to binding to algae is not accounted for. However, considering that the log K_{ow} is 2.34 (indicated in KOWWIN as experimentally determined) and the low adsorption potential of bentazone, (median $K_{f,oc}$ value of 25.2 L/kg) the results can be considered reliable with restrictions (Klimisch score of 2). The results can be used for classification purposes.

Hughes (1991a) performed a 14-day static lemna growth inhibition test with bentazone (purity of 53%) using duckweed (*Lemna gibba*) without following a specific guideline (Document IIA/ Section 8.6/06). Five concentrations were tested with the nominal test concentrations being 1, 2, 4, 8 and 16 mg/L. Control was included. All treatments were tested in triplicate. Test vessels were 500-mL glass Erlenmeyer flasks containing three plants with four fronds in 200mL synthetic 20x AAP nutrient medium. Temperature was maintained at 25±2 °C, and pH ranged 7.61-9.59. Vessels were incubated under continuous light (4198 - 5813 lumens/m^2). Growth was assessed by counting fronds on days 2, 4, 7, 9, 11 and 14. Analytical monitoring was performed at test start and end using GC with nitrogen-phosphorus detection. Actual bentazone concentrations ranged 88-96% and 57-79% of nominal at test start and end, respectively. Mean measured concentrations were <0.10, 0.794, 1.53, 3.06, 6.48 and 13.75 mg bentazone/L. Growth in control during 14 day exposure corresponded to a 61.3 x multiplication (736 fronds at test end). At test end, significantly reduced yield was found at the two highest test concentrations. The 14d-E_yC50 was reported to be 5.35 mg/L, and the 14d-NOE_yC 3.06 mg/L both based on mean measured test concentrations and impaired yield. The study was considered acceptable in the RAR. The dossier submitter calculated an E_yC10 of 2.9 mg/L based on the inhibition percentages given in Table B.9.2.1.1-07 of Annex 1. The dossier submitter notes that this study was conducted before the adaptation of OECD TG 221 (2006) and as such has some deviations. Test duration is with 14 days twice as long as specified by OECD TG 221, i.e. 7 days. However, as indicated in section I.2.3.2 of the CLP guidance exposure up to 14 days is considered acceptable. In this study frond number was the only assessed endpoint, without the addition of another variable such total frond area, dry weight or fresh weight. The effect concentrations are based on yield and not growth rate. Considering above, the results are preferably not used for classification purposes.

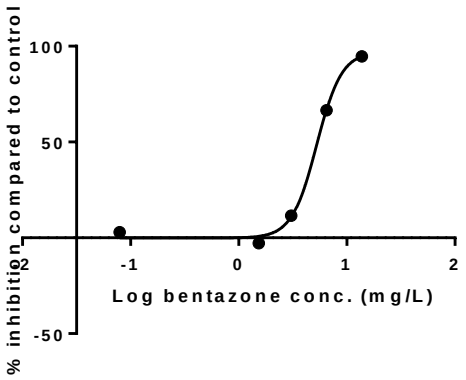


Figure 1. Dose response relationship of the 14-day static lemna growth inhibition study (Hughes, 1991a)

Hoffmann (2011b) performed a 7-day static lemna growth inhibition test with bentazone (purity of 100%) using duckweed (*Lemna gibba*) according to OECD TG 221 (Document IIA/ Section 8.6/01). Six concentrations were tested with the nominal test concentrations being 0.41, 1.23, 3.70, 11.1, 33.3, and 100 mg/L in triplicate. Control was included, and had six replicates. Test vessels contained three plants, i.e. two plants with four fronds and one plant with three fronds, in 160 mL 20x AAP medium. Temperature was maintained at 24-25°C, and pH ranged 7.48-8.45. Vessels were incubated under continuous light (8300 lux). On days 3, 5 and 7 number of fronds were counted, and observations with regard to chlorosis, necrosis, changes in plant size, shape and root growth were made. In addition the biomass based on dry weight was determined at test start and end. Analytical monitoring was performed at test start and end using HPLC-UV. Actual bentazone concentrations ranged 99.0-105.1% and 78.4-104.8% of nominal at start and end, respectively. 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. No morphological effects were observed up to 3.5 mg/L. At 11.1, 34.2 and 103.7 mg/L about two third of the fronds appeared smaller and one frond was necrotic from day 5. Effect concentrations based on yield and growth rate were reported, both based on the endpoints frond numbers as well as dry weight (see table below). The study was considered acceptable in the RAR. The

dossier submitter considers that the lowest values based on growth rate can be used for classification purposes, i.e. E_rC50 of 12.0 mg/L (dry weight), and E_rC10 of 3.2 mg/L (frond numbers), both based on mean measured test concentrations.

Table 16. Endpoints reported for *Lemna gibba* by Hoffmann (2011b)

Endpoint	**EC50 (95% CL) [mg/L; mean measured]**	**EC10 (95% CL) [mg/L; mean measured]**
Growth rate (frond numbers)	25.3 (20.5-31.5)	3.2 (1.8-4.7)
Growth rate (dry weight)	12.0 (10.5-13.7)	3.3 (2.4-4.1)
Yield (frond numbers)	9.1 (7.8-10.6)	3.5 (2.3-4.6)
Yield (dry weight)	7.1 (6.6-7.6)	3.2 (2.7-3.6)

Hoffmann (2011a) performed a 7-day static lemna growth inhibition test with bentazone-Na (purity of 91.9%) using duckweed (*Lemna gibba*) according to OECD TG 221 (Document IIA/ Section 8.6/02). Six concentrations were tested with the nominal test concentrations being 0.41, 1.23, 3.70, 11.1, 33.3, and 100 mg/L (corrected for purity) in triplicate. Control was included, and had six replicates. Test vessels contained three plants, i.e. two plants with four fronds and one plant with three fronds, in 160 mL 20x AAP medium. Temperature was maintained at 24-25°C, and pH ranged 7.48-8.47. Vessels were incubated under continuous light (8300 lux). On days 3, 5 and 7 number of fronds were counted, and observations with regard to chlorosis, necrosis, changes in plant size, shape and root growth were made. In addition the biomass based on dry weight was determined at test start and end. Analytical monitoring was performed at test start and end using HPLC-UV. Actual bentazone concentrations ranged 101.6-106.5% and 74.8-110.3% of nominal at start and end, respectively. 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 a 11.2-fold multiplication in seven days. Mean number of fronds after seven 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 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 1.12 mg/L. At 3.5 and 11.2 mg/L about one third of the fronds appeared smaller from day 5 on. At 35.9 and 103.9 mg/L two third of the fronds were smaller from day 5 on and the roots were smaller at test termination. Effect concentrations based on yield and growth rate were reported, both based on the endpoints frond numbers as well as dry weight (see table below). The study was considered acceptable in the RAR. The dossier submitter notes that the effect concentrations need to be corrected for sodium. The lowest corrected values based on growth rate are an E_rC50 of 17.0 mg/L (dry weight), and E_rC10 of 3.2 mg/L (dry weight), both based on mean measured test concentrations. The results can be used for classification purposes.

Table 17. Endpoints reported for *Lemna gibba* by Hoffmann (2011a)

Endpoint	**EC50 (95% CL) [mg/L; mean measured]**	**EC10 (95% CL) [mg/L; mean measured]**
Growth rate (frond numbers)	23.0 (20.0-26.4)	3.8 (2.7-4.9)
Growth rate (dry weight)	18.6 (16.0-21.5)	3.5 (2.5-4.6)
Yield (frond numbers)	9.8 (8.6-11.0)	3.2 (2.3-3.9)
Yield (dry weight)	8.6 (7.5-9.9)	2.5 (1.8-3.2)

In addition to the data presented in the RAR, studies that investigated the effects of bentazone on aquatic primary producers are also available in the public literature. The data provided in the RAR are sufficient for classification, however, for the completeness of the dossier literature data have been included below. A search using the CAS of bentazone yielded in EPA's ECOTOX database 287 endpoints for aquatic primary producers (accessed May 15th 2019), of which 22 endpoints concern (acute) EC/LC50 values and 75

(chronic) NOEC values. The corresponding studies were conducted with either bentazone or the formulated product. The studies using the formulated product were not further considered as the observed effects cannot be contributed to bentazone alone. Effect concentrations that were considerably less critical than the values reported in the RAR were also not further considered. This resulted in five studies, which are discussed below.

Cedergreen and Streibig (2005) performed a 48-hours static algal growth inhibition study with bentazone (purity >90%) using the green alga *Pseudokirchneriella subcapitata* in accorordance with ISO guideline 8692:2012. Eight concentrations were tested in duplicate. Nominal test concentrations were not specified. Control was included, and had six replicates. Test vessels and medium were not specified. Initial cell density was 1x 10^5 cells/mL. Vessels were incubated under continuous light 80 PAR photons µmol/m^2.s. pH was determined at start and test end and ranged 8.0-8.5. Temperature was 22 °C. Hardness were not reported. Chlorophyll content was determined at 0, 24 and 48 hours using a fluorometer and served as a proxy of algal density (instead of counting algal cells). The algal growth rate was reported to be exponential. An E_rC10 of 1.77±1.11 mg/L and an E_rC50 of 13.6±2.29 mg/L both based on nominal test concentrations, were reported. The Dossier Submitter notes that the validity of the study cannot be determined as the biomass of the control was not specified (minimal multiplication factor should be 16). Also no information is given with regard to the variability between control replicates (CV of average specific growth rate should be ≤7%) and if the control growth rate remained constant over time (CV section-by-section specific growth rates should be ≤35%). Effect concentration is based on nominal concentrations. Minor shortcomings are that the initial cell density was above the specified range of 5 x 10^3-10^4 cells/mL, and that the exposure duration was 48 and not 72 h. Considering above, the reliability of the study cannot be assessed and the data are assigned a Klimisch score of 4 (= unassignable). Munkegaard et al. (2008) used exactly the same test setup as Cedergreen and Streibig (2005) and reported an E_rC50 of 0.051 ± 0.004 mg/L for *Pseudokirchneriella subcapitata* based on nominal test concentrations. The reliability of this study could also not be assessed and the data are assigned a Klimisch score of 4 (= unassignable). These data are not used for classification purposes.

Macedo et al. (2008) performed a 72-hours static algal growth inhibition study with bentazone (purity not reported) using the marine diatom *Skeletonema costatum* according to ISO guideline 8692:2012. Eight concentrations were tested in duplicate with the nominal test concentrations being 0.70, 1.41, 2.81, 5.62, 11.25, 22.50, 45.00 and 90.00 mg/L. Control was included, and also had three replicates. Test vessels were 250-mL glass Erlenmeyer with 150 mL F/2 medium (salinity 31). Initial cell density was not specified. Vessels were incubated under light 80 µE/(m^2.s) with light/dark cycle of 12/12h. Temperature was 20±2°C. Hardness was not reported. pH was 8.05±0.01 at test start, and increased by the end to 9.28±0.08. Algal growth (chlorophyll a fluorescence and cell density) and maximum quantum yield of photosystem II (Fv/Fm) were monitored after 0, 6, 12, 24, 48 and 72 h. Algal growth rates were calculated based on fluorescence measurements and algal growth inhibition based on the growth rates obtained for bentazone treated cultures contrasted to those obtained for the controls. A LOEC of 22.5 mg/L, corresponding to NOEC of 11.25 mg/L, and an EC50 of 24.0 mg/L were reported based on nominal test concentrations. The Dossier Submitter notes that it is unclear if validity criteria were met, i.e. minimal multiplication factor of 16 in control; CV of average specific growth rate in replicate control cultures of ≤7%, and CV section-by-section specific growth rate in control of ≤35%. Furthermore, control should have six and not three replicates, and there were no analytical measurements. Considering above, the reliability of the study could also not be assessed and the data are assigned a Klimisch score of 4 (= unassignable). These data are not used for classification purposes.

Hourmant et al. (2009) performed a 72 h non-guideline algal growth inhibition study with bentazone (purity not reported) using the marine diatom *Chaetoceros gracilis*. Five concentrations were tested in three to six replicates with the nominal test concentrations being 1, 10, 50, 80 and 100 µg/L. Control was included. Test vessels were 1-L glass bottles with 500 mL enriched sea water. Initial cell density was 0.2-1 x 10^4 cells/mL. Bottles were incubated under light 45 PAR photons µmol/(m^2.s) with photoperiod of 16h. Temperature was 18±2 °C. Hardness and pH were not reported. Analytical measurements were conducted in sub-samples. Growth was determined by counting cells. A NOEC of 10 mg/L and an EC50 of 150 mg/L were reported based on growth rate and expressed as nominal test concentrations. The Dossier Submitter notes that no details were reported on the performance of the control, and it can not be determined if exponential growth took place. Overall, the reliability of the study could not be assessed and the data are assigned a Klimisch score of 4 (= unassignable). These data are not used for classification purposes.

Cedergreen and Streibig (2005) performed a 7-day static lemna growth inhibition test with bentazone (purity >90%) using common duckweed (*Lemna minor*) in accordance with ISO guideline 20079:2005. Eight concentrations were tested in triplicate. Nominal test concentrations were not specified. Control was included, and had six replicates. Test was conducted in six-well tissue-culture test plates. In each well, one *L. minor* frond was transferred to 10 ml K-medium (Maeng and Khudairi, 1973) with pH 5 at 24°C. Total frond area was measured at start and test end, and was used to calculate the relative growth rate. Water quality and analytical measurements were not performed. An E_rC10 of 1.14±2.20 mg/L and E_rC50 of 2.56±1.45 mg/L were reported. The Dossier Submitter notes that the validity of the study cannot be determined as the doubling time in the control has not been reported. This should be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 per day. Other shortcomings were: test vessels were not made of inert material, test volume was 10 mL instead of recommended 100 mL, number of fronds should have been 9 to 12 (OECD TG 221), and not 1 per test vessel, there were no water quality or analytical measurements, endpoints such as number of fronds and dry/fresh weight were not considered. Effect concentrations are based on nominal concentrations. Considering above, the reliability of the study cannot be assessed and the study is assigned a Klimisch score of 4 (= unassignable). Munkegaard et al. (2008) used exactly the same test setup as Cedergreen and Streibig (2005) and reported an E_rC50 of 2.94±0.22 mg/L for *Lemna minor* based on nominal test concentrations. The reliability of this study could also not be assessed and the data are assigned a Klimisch score of 4 (= unassignable). These data are not used for classification purposes.

From the above summaries it can be concluded that there is one reliable algal inhibition study yielding in an ErC50 of 32.8 mg/L and an ErC10 of 9.7 mg/L (nominal, with analytical verification of replicate without algae). For the duckweed there are three reliable studies, of which one is preferably not used as the effect concentrations are based on yield, and not growth rate. The remaining two studies are identical experimental setup, except that one study was conducted with bentazone (Hoffmann, 2011b) and one with bentazone-Na (Hoffmann, 2011b) . The effect concentrations reported by the latter two studies hardly differ with the values reported by Hoffmann (2011b) being an E_rC50 of 12.0 mg/L (dry weight), and E_rC10 of 3.2 mg/L (frond numbers) (both mean measured), and the values reported by Hoffmann (2011a) being an E_rC50 of 17.0 mg/L (dry weight), and E_rC10 of 3.2 mg/L (dry weight), also both based on dry weight. As there are less than four reliable values for duckweed, the lowest values as reported by Hoffmann (2011b) will be used for classification purposes

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data available.

11.6 Long-term aquatic hazard

Table 18: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks[1]	Reference
Fish					
Fish early life stage toxicity test Flow-through OECD TG 210; US EPA-FIFRA 72-4; EPA-OPPTS 850.1400	Fathead minnow (*Pimephales promelas*)	Bentazone-Na (BAS 351 H-Na) Purity: 91.9%	35d-NOEC: 9.0 mg/L (corrected for sodium)	mean measured Klimisch score of 1 **Key data**	Anonymous, 2011b
Fish, juvenile growth test Flow-through	*Oncorhynchus mykiss*	Bentazone 480 g/L SL (formulated product)	21d-NOEC: 5.91 mg/L (expressed as	nominal (measured concentrations did not	Document IIIA/ Section 10.2.5/01

OECD TG 215		Purity: 480 g/L (~40% bentazone)	bentazone)	remain constant) Klimisch score of 3	
Aquatic invertebrates					
Daphnia reproduction study Semi-static OECD TG 211	*Daphnia magna*	Bentazone 480 g/L SL (formulated product) Purity: 480 g/L (~40% bentazone)	21d-NOEC: 32 mg/L (expressed as bentazone)	nominal (actual conc. ~100% of nominal) Klimisch score of 2 **Key data**	Jatzek, 1989a
Daphnia reproduction study Semi-static OECD TG 211	*Daphnia magna*	Basagran (BAS 351 32 H; formulated product) Purity: 40.4% bentazone	21d-NOEC: 101 mg/L (expressed as bentazone)	nominal (actual conc. ~100% of nominal) Klimisch score of 2 **Supportive data**	Migchielsen, 2001
Algae or other aquatic plants					
Algal growth inhibition static OECD TG 201	*Pseudokirchneriella subcapitata* (current name *Raphidocelis subcapitata*)	Bentazone (BAS 351 H) Purity: 98.4%	72h-E_bC_{10}: 9.7 mg/L (corrected for purity)	nominal (actual conc. ~100% of nominal, but determined in replicate without algae) Klimisch score of 2 **Supportive data**	Document IIA/ Section 8.4/01
Algal growth inhibition static ISO guideline 8692:2012	*Pseudokirchneriella subcapitata*	Bentazone Purity: >90%	48h-E_rC_{10}: 1.8±1.1 mg/L	nominal Klimisch score of 4	Cedergreen and Streibig, 2005
Algal growth inhibition static ISO guideline 8692:2012	*Skeletonema costatum*	Bentazone Purity: not reported	NOEC: 11.3 mg/L	nominal Klimisch score of 4	Macedo et al., 2008
Algal growth inhibition	*Chaetoceros gracilis*	Bentazone Purity: not reported	NOEC: 10 mg/L	nominal Klimisch	Hourmant et al., 2009

static non-guideline				score of 4	
Lemna growth inhibition static non-guideline	*Lemna gibba*	Bentazone (BAS 351 H) Purity: 53.0%	14d- E_yC_{10}: 2.9 mg/L	mean measured Klimisch score of 2 **Supportive data**	Document IIA/ Section 8.6/06
Lemna growth inhibition static OECD TG 221	*Lemna gibba*	Bentazone Purity: 100%	**7d-E_rC10: 3.2 mg/L (frond numbers)**	mean measured Klimisch score of 1 **Key data**	Document IIA/ Section 8.6/01
Lemna growth inhibition static OECD TG 221 OPPTS 850.4400 (draft)	*Lemna gibba*	Bentazone-Na Purity: 91.9%	**7d-E_rC10: 3.2 mg/L (dry weight)**	mean measured Klimisch score of 1 **Key data**	Document IIA/ Section 8.6/02
Lemna growth inhibition static ISO guideline 20079:2005	*Lemna minor*	Bentazone Purity: >90%	7d-E_rC10: 1.1±2.2 mg/L (frond area)	nominal Klimisch score of 4	Cedergreen and Streibig, 2005

[1] Studies indicated as key data refer to the most conservative study; other reliable experimental information is referred to as supportive data.

11.6.1 Chronic toxicity to fish

The RAR contains one long-term fish toxicity test with bentazone.

Anonymous (2011b) performed a 35-day limit test under flow-through conditions with bentazone-Na (purity of 91.9%) using fathead minnow (*Pimephales promelas*) according to OECD TG 210 (1992) and US EPA-FIFRA 72-4, EPA-OPPTS 850.1400 (Document IIA/ Section 8.2.4/01). One nominal test concentration of 10 mg/L (after correction for purity) and a control were tested in four replicates. Each replicate consisted of 25 eggs (< 3.5 h old; blastodisc cleavage stage) that were incubated in a glass vessels placed in flow-through chamber containing 1.7-L test solution at a flow-rate of 2.25 L/h. After hatching, juveniles were transferred into 9-L stainless steel aquaria with a flow rate of 9.0 L/h. Medium was non-chlorinated charcoal filtered drinking water diluted with deionised water, total hardness 95 - 104 $CaCO_3$ mg/L, conductivity 238 - 257 µS. Test temperature ranged 24.7-26.1 ºC, pH 7.7-8.1. Slight aeration was applied from day 18 till test end because of low oxygen (≥ 64 % saturation). Feeding was applied from day 5 (end of the hatch) till test end consisting of live brine shrimp nauplii and a fine commercial fish diet. Observations included: egg mortality until hatch, larval mortality and signs of toxicity/abnormal behaviour of larvae. Latter observations were conducted daily until the end of swim-up, and afterwards weekly. Analytical monitoring was performed at days 0, 9, 16, 23, 30, and 35 using HPLC-UV. LOQ 1 mg/L. Mean measured concentration was 9.83 mg/L (98.3% of nominal). There were no significant effects reported, except for fish length at test end. Hatching amounted to 97% (92-100%) in the control and 98% (96-100%) in the treatment. Hatching started on day 3 and was completed by day 5. Larval survival (from hatch until end of swim-up) was 96-100% in the treatment. Start and end of swim up was equal in control and treatment. Juvenile survival in the control was

94% (91-100%) and 91% (84–96%) in the treatment. Mean overall survival was 91% (84-96%) in the control and 88% (84-92%) in the test group. No abnormalities were observed in control or treatment. Mean body weight and length were not statistically decreased as compared to control. Length was significantly increased as compared to control. The registrant concluded that as an increase is considered not an adverse effect, this effect was not taken into account for determination of the NOEC. The 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-Na. Study was considered acceptable in the RAR. The dossier submitter notes that dissolved oxygen levels were not reported, while this is a relevant water quality parameter. This is not considered a major issue, considering that the RMS concluded in the RAR that water quality parameters were within accepted limits. The dossier submitter further notes that the mean measured NOEC should be corrected for sodium, resulting in a 35d-NOEC of 9.0 mg/L. The results can be used for classification purposes.

In addition, the RAR contains a fish, juvenile growth test (OECD TG 215) with the formulated product Bentazone 480 g/L SL (purity 480 g/L) that contains bentazone (~40%) (Document IIIA/ Section 10.2.5/01). The 28-day flow-through test was conducted with the rainbow trout *Oncorhynchus mykiss* and a NOEC of 5.91 mg/L expressed as bentazone is reported in the RAR based on nominal concentrations. This study was considered unreliable and not acceptable during the renewal (see Annex 1 for detailed RAR assessment). Main issues were unstable exposure concentrations that declined till day 14, and were equal to or above initial concentrations at days 21 and 28. Furthermore effects were reported that were not attributable to bentazone at the lower test concentrations. Overall, the RMS concluded during renewal that the study is unreliable. The Dossier Submitter agrees (Klimisch score of 3), Furthermore, a reliable long-term fish toxicity test with the active substance bentazone is available. The results of the fish, juvenile growth test with formulated product are not used for classification purposes.

11.6.2 Chronic toxicity to aquatic invertebrates

There are no studies available in the RAR that investigated long-term toxicity of bentazone to aquatic invertebrates. The RAR does contain two daphnia reproduction studies that were conducted with formulated product that contain bentazone.

Jatzek (1989a) performed a 21-day daphnia reproduction study under semi-static conditions with the formulated product BAS 351 32 H (Basagran; consisting for 40.4% of bentazone) using *Daphnia magna* according to OECD TG 211 (Document IIIA/ Section 10.2.6/01). Eight nominal test concentration of 3.906, 7.81, 15.6, 31.3, 62.5, 125, 250 and 500 mg BAS 351 32 H/L, and a control were tested in ten replicates. Each replicate consisted of 1 daphnid neonate incubated in a 100-mL test vessels containing 50 mL test solution. Medium was artificial fresh water: total hardness 2.65-2.78 mmol/L; conductivity 620 µS/cm; pH 7.76-8.12; oxygen content: 8.16 - 9.49 mg/L; Test temperature ranged 18.85 - 20.85 ºC. No aeration. Daily feeding with green algae *Scenedesmus subspicatus*. Parent mortality and reproduction were observed three times a week. Analytical monitoring was performed at days 0, 2, 9, 11, 18 and 21. Mean recoveries of BAS 351 32 H ranged 97-105% of nominal in fresh solutions, and 96-113% of nominal before renewal. No mortality in the control. Mortality only observed 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 amounted to 87 in the control and 90, 82, 83, 81, 102 , 96, 88, and 39 for the 3.906, 7.81, 15.6, 31.3, 62.5, 125, 250 and 500 mg/L treatments, respectively. Day of first brood was day 9 for all test item concentrations and the control. The NOEC for reproduction was set as 250 mg formulation/L, equivalent to 101 mg bentazone/L. Study was considered acceptable in the RAR. The dossier submitter notes that number of renewals as not reported, that the analytical methodology was not specified, that the LOQ was not specified, and that the NOEC is expressed based on nominal test concentrations that have been analytically verified. Study is considered reliable and the results can be used for classification purposes.

Migchielsen (2001) performed a 21-day daphnia reproduction study under semi-static conditions with the formulated product BENTAZONE 480 g/L SL (purity 480 g/L; batch 0203005; density 1.1916 g/mL) using *Daphnia magna* according to OECD TG 211 (Document IIIA/ Section 10.2.6/01). Renewal of test solution three times a week. Five nominal test concentration of 25, 45, 80, 145 and 250 mg BENTAZONE 480 g/L SL/L were tested in ten replicates, and the control in twenty replicates. Each replicate consisted of 1 daphnid (neonate <24 h old) incubated. Medium was M7 medium: total hardness 250 mg/L $CaCO_3$. Test temperature

ranged 19.4 – 21.0 ºC; pH: 7.4-8.3 in fresh media, and 7.5-8.6 in old media; oxygen content: 7.3-9.4 mg/L in fresh media and 8.0-9.9 mg/L in old media. Parent mortality, length and reproduction parameters were observed every working day. Analytical monitoring was performed at start (fresh medium) and end (old medium) of a renewal period, and analysed by HPLC-UV. LOQ was 25 mg bentazone/L. Mean measured concentrations ranged from 96 - 102% of nominal. Parental mortality was observed in all treatments and amounted to 20% in the control and 10, 40, 60, 30 and 100% in the 25, 45, 80, 145 and 250 mg/L treatments, respectively. The RAR notes that the registrants concluded that the mortality of the parental daphnids was not treatment related, but also that no reason for the observed mortality was given. Average parent body length was 5.47 mm for the control and ranged 5.26 to 5.58 mm for the treatments. The highest treatment had a reduction of 4.3% which was significantly lower than the control according to Bonferroni-t-test but not according to Tukey's test. The brood on day 8 was 7.6 living new-born per vessel for the control, and 4.6, 7.4, 9.6, 8.7 and 0.8 for the 25, 45, 80, 145 and 250 mg/L treatments, respectively. The cumulative offspring per parent was 187 for the control and 197, 179, 204, 172, and 92 for the 25, 45, 80, 145 and 250 mg/L treatments, respectively. A significant reduction of reproduction as observed at the two highest concentrations. The NOEC for reproduction was set at 80 mg BENTAZONE 480 g/L SL, corresponding to 32 mg bentazone/L. Study was considered acceptable in the RAR. The dossier submitter notes that the purity of the formulation was not reported, but from section 2.1.3 of the RAR it is clear that both Bentazone 480g/L SL (Agrichem) and Basagran SL (BAS 351 32 H; BASF) contain 480 g bentazone/L corresponding to purity of ~40%. The mortality in the control is 20% which is still acceptable according to OECD TG 211 (should not exceed 20%). Study is considered reliable and the results can be used for classification purposes.

From the above summaries it can be concluded that there are two reliable daphnia reproduction studies. Both studies were conducted with formulated products, and as such it cannot be excluded that effects can at least partially be attributed to other constituents of the formulations. However, since there are no long-term toxicity studies with aquatic invertebrates exposed to just bentazone, the available data can be used, representing a worst-case approach. The studies report 21-day NOECs of 101 and 32 mg/L when expressed as bentazone. Both values are based on nominal test concentrations that have been analytically verified. As there are less than four reliable values for daphnids (and they have been generated with formulations that might not be exactly the same in composition), the lowest value as reported by Migchielsen (2001) will be used for classification purposes.

11.6.3 Chronic toxicity to algae or other aquatic plants

Please see paragraph 11.5.3.

11.6.4 Chronic toxicity to other aquatic organisms

No data available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Experimental data are available for algae, aquatic plants, aquatic invertebrates and fish. For algae, one reliable study is available reporting a 72h-E_rC50 of 32.8 mg/L (growth rate) based on nominal concentrations, but with analytical verification in replicates without algae. For the aquatic plant *L. gibba* there are three reliable studies of which two reported effect concentrations based on growth rate. The lowest acute effect concentration for aquatic plants is a 7d-E_rC50 of 12 mg/L (growth rate) based on mean measured test concentrations. For aquatic invertebrates and fish only limit tests are available where no detrimental effects were observed during acute exposure. The lowest tested values were a 48h-EC50 of >98.4 mg/L for the aquatic invertebrate *D. magna* and a 96h-LC50 of >94 mg/L for the fish *Lepomis macrochirus*.

From these data is clear that aquatic plants are the most sensitive species, with a 7d-E_rC50 of 12 mg/L. Therefore, in accordance with table 4.1.0(a) (according to CLP guidance V4.1 June 2015, p. 503-505) and

considering that the lowest acute effect concentration is above 1 mg/L, bentazone is not to be classified for acute aquatic toxicity.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

The biodegradation potential of bentazone has not been evaluated using a ready biodegradability test. In a biodegradability screening study where sediment was used as inoculum instead of activated sludge, and where exposure duration was 117 days instead of 28 days primary degradation was not observed (0% at 10 and 20 mg/L). Low biodegradability is supported by QSAR estimations with BIOWIN estimating bentazone as not readily biodegradable. Simulation data for the surface water compartment is not available. Degradation in water/sediment systems is very slow with DT50 values for the sediment of 568-595 days at 20°C, and total system DT50 of 688-940 days at 20°C. Correction to an EU relevant temperature of 12°C would result in DT50 for sediment of 1205-1263 days, and DT50 total system of 1460-1497 days. Bentazone can degrade to *N*-methylbentazone (max 13%) in water-sediment systems, with the formation being reversible (see sections 11.1 and 11.5). The DT50 values reported for soil concern dissipation and not degradation, as non-extractable residues (NER) were not accounted for. The available soil degradation data show that bentazone is slowly degraded in soil, and that determination of degradation DT50 values is hampered by adsorption of bentazone to soil. Overall based on this information, it can be concluded that bentazone should be considered as not rapidly biodegradable for classification purposes.

The bioaccumulation potential of Bentazone was studied in *Lepomis macrochirus* using radiolabelled test material. BCF values of 0.4, 2.2, and 1.4 L/kg were reported for edible, non-edible and whole body, respectively. These values were not corrected for growth or normalized to 5% lipid content. That said, the threshold of 500 L/kg is not expected to be approached. The highest log D_{ow} of 1.54 determined at pH 4, which can be considered representing worst-case for environmental conditions, is also far below the threshold of log Kow ≥4. Therefore, bentazone is considered to have a low potential for bioaccumulation.

For bentazone, reliable chronic studies are available for three trophic levels: algae/aquatic plants, aquatic invertebrates and fish. For algae, a 72h-E_rC10 of 9.7 mg/L (growth rate) is available that is based on nominal concentrations, but with analytical verification in replicates without algae. For the aquatic plant *L. gibba* there are three reliable studies of which two reported effect concentrations based on growth rate. The lowest chronic effect concentration for aquatic plants is a 7d-E_rC10 of 3.2 mg/L (growth rate) based on mean measured test concentrations. For aquatic invertebrates, a 21d-NOEC of 32 mg/L based on nominal concentrations, but with analytical verification is available. For fish, a 35d-NOEC of 9.0 mg/L based on mean measured test concentrations is available.

From these data, it is clear that aquatic plants are the most sensitive species, with a 7d-E_rC10 of 3.2 mg/L. In accordance with table 4.1.0(b(i)) (according to CLP guidance V4.1 June 2015, p. 503-505) and considering that the lowest chronic effect concentration is above 1 mg/L, bentazone is not to be classified for chronic aquatic toxicity.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Conclusions on classification and labelling for environmental hazards of bentazone.

	CLP regulation	
	Classification	M-factor
Resulting harmonised classification.	-	-

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not evaluated in this dossier

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Not evaluated in this dossier

12.1.2 Comparison with the CLP criteria

Not evaluated in this dossier

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not evaluated in this dossier

13 ADDITIONAL LABELLING

[If relevant, please justify here the reason for supplemental hazard information in accordance with Annex II of the CLP Regulation.]

14 REFERENCES

A full reference list for all the studies from the DAR are included in Annex I. In addition, the following references were used in this CLH report.

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15 ANNEXES

The study summaries from the Renewal Assessment Report for Bentazone, January 2015, have been included in Annex I.